REMARKS

Reconsideration of the application in view of the above amendments and following remarks is requested. Claims 1, 2 and 8 are currently pending. Claims 3 and 4 have been canceled. Applicants reserve the right to prosecute cancelled subject matter in later filed applications and in no way are disclaiming any subject matter. Applicants assert that no new matter is added by the present amendment.

ELECTIONS/RESTRICTIONS

The Examiner has acknowledged Applicants election of Group I (claims 1-4 and 8) in the December 12, 2005 Response. However, in the November 18, 2005 Restriction Requirement, Applicants note that while Group IV was rightfully directed to the subject matter of claims 12 and 13, it was characterized as being directed to claims 9-11. Thus, Applicants assert that Section 4 of the Disposition of Claims should state that "Claims 1-13 are pending in the application" and Section 4a should state that "Of the above claims, 5-7 and 9-13 are withdrawn from consideration." Applicants respectfully request correction.

CLAIM OBJECTIONS

Claim 3 has been rejected under 37 CFR 1.75 as a duplicate of claim 4. Accordingly, Applicants have cancelled claim 4.

THE §101/§112, FIRST PARAGRAPH REJECTIONS

Claims 1-4 and 8 are rejected under 35 U.S.C. §101 and §112, first paragraph, alleging that the claimed invention is not supported by either a specific and substantial asserted utility or a well-established utility.

Applicants traverse. The present invention is based on the discovery of a novel class II cytokine, which Applicants designated Zcyto10 (also known as IL-20). Specifically, Zcyto10 is described as a four-helix-bundle cytokines such as those found within the interferon/IL-10 class:

> **It is believed that Zcyto10 is of a member of the IL-10 subfamily of cytokines. Other members of this group include MDA-7, IL-19, and KFF.**

Id at pg. 9, lines 4-5. Concurrent with the discovery of Zcyto10, Applicants identified ZcytoR7 (IL-20RA) as a novel cytokine receptor: "ZcytoR7, like all known class II receptors except for interferon-alpha/beta receptor alpha chain, has only a single class II CRM in its extracellular domain. ZcytoR7 appears to be a receptor for a helical cytokine of the interferon/IL-10 class." *See e.g.* US Patent No. 5,945,511. As stated in the present Application, it was subsequently confirmed that Zcyto10 bound to a receptor complex comprising ZcytoR7. *See e.g.*, Blumberg et *al.*, Cell, 104:9-19, (2001) (copy enclosed).

The Examiner has alleged that the present Application "does not disclose a specific and substantial biological role of this protein or its significance...There is no biological activity, phenotype, disease or condition, or aay other specific feature that is disclosed as being associated with the IL-20 polypeptide."

Applicants strongly disagree. Applicants were the first to discover IL-20's biological function and its role in skin disorders. As stated in the present Application, IL-20 has been recognized to be involved with skin disorders: "Zcyto10 polypeptides can also be used to treat a number of skin conditions..., for example eczema, psoriasis or dry skin conditions in general or as related skin attentions." *See e.g.*, Specification at pg. 31, line 35 through pg. 36, line 3; *see also*, Blumberg et *al*. To support this utility, Applicants describe the **phenotypic effect** that IL-20 transgenic mice present in Example 5. Specifically, these transgenic mice exhibit skin that was "tight and wrinkled" while the "skin of the zcyto10 expressing pups, *particularly those mice which had a* ***high*** *expression level of Zcyto10 tended to be thicker than the non-expressing pups.*" *Id* at pg. 39. Applicants strongly assert that the present Application has clearly demonstrated that IL-20 has a biological role or function in skin conditions such as psoriasis. Thus, polynucleotides encoding the IL-20 protein would have clear utility.

Applicants have also enclosed a number of additional references that also support the asserted and specific utility that is clearly stated in the present Application. Rich and Kupper, Current Biology, 11:R531-R534 (2001) (copy enclosed), citing Applicants transgenic data summarized above, state that "Biological studies of IL-20 revealed that it has an important role in promoting hyperproliferation of keratinocytes and thereby modulating inflammation in the skin." Another reference, Volk *et al.*, TRENDS in Immunology, 22(8):414-417 (2001) (copy enclosed), ***also citing Applicants transgenic data***, state that IL-20's "selective pro-inflammatory activities make [it] interesting new candidates for research and drug development." Rich, B. E., Expert Opin. Ther. Targets, 7(2):165-174 (2003) (copy enclosed) states that "IL-20 signaling appears to be a prominent component of cutaneous inflammation." Rich goes on to state that "The apparently specialized role of IL-20 signaling in cutaneous tissue may present an opportunity to create pharmaceutical interventions that selectively mitigate inflammatory processes in the skin while sparing inflammation in other tissues." *Id.* Thus, as clearly stated in the present Application, IL-20 polypeptides would be useful in generating therapeutics for treating such skin conditions.

Accordingly, Applicants assert that the polynucleotides of the present invention encode an IL-20 polypeptide that has a recognizable biological function which would be understood and appreciated by one skilled in the art upon reading the present Application. Accordingly, reconsideration and withdrawal of the rejection under 35 U.S.C. §101 and §112, first paragraph is respectfully requested.

The Examiner has also stated that Applicants "asserted utility" for Zcyto10 "to promote wound healing" is not specific or substantial because the Specification does not disclose a specific and substantial biological role for this protein or its significance.

Applicants strongly disagree. Applicants respectfully remind the Examiner that they "need only provide one credible assertion of specific and substantial utility." *See e.g.* M.P.E.P. §2107(II). Applicants strongly assert that they have in fact provided a credible, specific and substantial utility: **Zcyto10 is useful in promoting wound healing**. As stated in the M.P.E.P. §2107.01(I)(A) a "specific utility" is one where an Applicant "discloses a specific biological

activity" and "reasonably correlates that activity to a disease condition". As stated above, Applicants have indeed disclosed a "specific and substantial" biological activity for Zcyto10: to promote wound healing. Moreover, in Example 4, Applicants specifically disclose that Zcyto10 expression is up-regulated in wounded skin. Accordingly, Applicants assert that they have in fact disclosed a "specific utility": Applicants disclosed that Zcyto10 is upregulated in wounded skin" (*i.e.* a "specific biological activity") and thus would be useful in the treatment of burns or to promote wound healing (*i.e.* "reasonably correlates that activity to a disease condition").

The Examiner has also stated that the asserted utility is not presented in a "ready-to-use, real-world application" and, as such, "is not substantial."

Again, Applicants disagree. Applicants have also described to one skilled in the art how to use Zcyto10 for the treatment of such a burn or a wound. *See e.g.* Specification at pg. 34, lines 11-27. Applicants assert that one skilled in the art would easily recognize that the Zcyto10 would be useful to promote wound healing and how to use the same to promote such wound healing.

Furthermore, Applicants assert that the M.PE.P. §2107.01(I)(B) specifically states that Office personnel must be careful not to interpret the phrase "immediate benefit to the public" or other similar phrases to mean that products based on the claimed invention must be "currently available" in order to satisfy the utility requirement, but rather "***any reasonable use that an Applicant has identified that can be viewed as providing a public benefit should be accepted as sufficient.***" Further, the Courts have repeatedly found that the *mere identification* of a pharmacological activity that is relevant to the asserted use provides such "an immediate benefit to the public" and thus satisfies the utility requirement. *See e.g.* M.P.E.P. §2107.01(III)(A); *see also Nelson v. Bowler,* 626 F.2d 853, 856, 206 USPQ 881, 883 (CCPA 1980), which states:

> **Knowledge of the pharmacological activity of any compound is obviously beneficial to the public.** It is inherently faster and easier to combat illnesses and alleviate symptoms when the medical profession is armed with an arsenal of chemicals having known pharmacological activities. Since it is crucial to provide researchers with an incentive to disclose pharmacological activities in as many compounds as possible,

> **we conclude that adequate proof of any such activity constitutes a showing of practical utility.**

In addition, the Courts have also found utility for therapeutic inventions that were at very early stages in development, based solely on the claimed biological activity of the compound:

> We perceive no insurmountable difficulty, under appropriate circumstances, **in finding that the first link in the screening chain, *in vitro* testing, may establish a practical utility for the compound in question.** Successful *in vitro* testing will marshal resources and direct the expenditure of effort to further *in vivo* testing of the most potent compounds, thereby providing an immediate benefit to the public, analogous to the benefit provided by the showing of an *in vivo* utility.

See e.g. In re Brana, 51 F.3d 1560, 34 USPQ2d 1436 (Fed. Cir. 1995). Accordingly, an Applicant is not required to demonstrate that a claimed invention is a fully effective drug for humans. *See e.g.* M.P.E.P. §2107.01(III).

Applicants assert that Zcyto10 has a specific and substantial asserted utility that would be easily recognizable, and understood and appreciated by one skilled in the art upon reading the present Application. The utilities of the claimed invention described above provide immediate benefit to the public. That is all that is required under 35 U.S.C. §101. Accordingly, Applicants respectfully request consideration and withdrawal of the present rejections under 35 U.S.C. §101 and §112.

THE §112, FIRST PARAGRAPH REJECTIONS FOR WRITTEN DESCRIPTION AND ENABLEMENT

The Examiner has rejected claims 1 and 2 under 35 USC § 112, first paragraph, as failing to comply with the written description and enablement requirements.

Applicants have amended claims 1 and 2 so that they no longer recite the percent identity language. Accordingly, Applicants believe that the present rejections under 35 U.S.C. §112, first paragraph are now moot.

THE §112, SECOND PARAGRAPH REJECTION

Claim 8 is rejected under 35 USC § 112, second paragraph, as failing to set forth the subject matter which applicants regard as their invention.

Applicants have amended claim 8 as suggested by the Examiner. Accordingly, Applicants believe that the present rejection is now moot.

CONCLUSION

On the basis of the above amendments and remarks, Applicants believe that each rejection has been addressed and overcome. Reconsideration of the application and its allowance are requested. If for any reason the Examiner feels that a telephone conference would expedite prosecution of the application, the Examiner is invited to telephone the undersigned at (206) 442-6558.

Respectfully Submitted,

Shelby J. Walker

Shelby J. Walker
Registration No. 45,192

Enclosures:

Blumberg et *al.*, <u>Cell</u>, 104:9-19, (2001)
Rich and Kupper, <u>Current Biology</u>, 11:R531-R534 (2001)
Volk *et al.*, <u>TRENDS in Immunology</u>, 22(8):414-417 (2001)
Rich, B. E., <u>Expert Opin. Ther. Targets</u>, 7(2):165-174 (2003)

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Interleukin 20: Discovery, Receptor Identification, and Role in Epidermal Function

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Summary

A structural, profile-based algorithm was used to identify interleukin 20 (IL-20), a novel IL-10 homolog. Chromosomal localization of IL-20 led to the discovery of an IL-10 family cytokine cluster. Overexpression of IL-20 in transgenic (TG) mice causes neonatal lethality with skin abnormalities including aberrant epidermal differentiation. Recombinant IL-20 protein stimulates a signal transduction pathway through STAT3 in a keratinocyte cell line, demonstrating a direct action of this ligand. An IL-20 receptor was identified as a heterodimer of two orphan class II cytokine receptor subunits. Both receptor subunits are expressed in skin and are dramatically upregulated in psoriatic skin. Taken together, these results demonstrate a role in epidermal function and psoriasis for IL-20, a novel cytokine identified solely by bioinformatics analysis.

Introduction

We describe the use of EST database mining in the discovery of interleukin-20 (IL-20), a novel IL-10-related cytokine. The IL-10 protein family includes IL-10 (Vieira et al., 1991), IL-19 (Rosen and Kenny, 1999), MDA-7 (Jiang et al., 1995), IL-TIF (Dumoutier et al., 2000a), and AK-155 (Knappe et al., 2000). The biological function of IL-10 itself has been explored in depth. IL-10 mediates its activity through a receptor which is composed of IL-10Rα and IL-10Rβ (CRF2-4; Kotenko et al., 1997). These receptor subunits form a heterodimeric structure similar to that found in interferon and other class II cytokine receptors. IL-10 regulates the function of both lymphoid and myeloid cells, and its immunosuppressive effects involve inhibition of proinflammatory cytokine synthesis by T cells, monocytes, and macrophages. IL-10 can also act as an immunostimulant by increasing thymocyte, mast cell, and B cell proliferation (Moore et al., 1993). Potential actions of IL-10 in a variety of cutaneous disorders were recently reviewed, including a therapeutic role for IL-10 as an anti-inflammatory cytokine in psoriasis (Asadullah et al., 1999).

We discovered IL-20 utilizing a bioinformatics algorithm designed to identify helical cytokines. Using a multifaceted approach, we have elucidated a biological function and disease association for the IL-20 protein. Results from TG overexpression of IL-20 in mice and in vitro assays using recombinant IL-20 protein led us to the discovery of an IL-20 receptor. This heterodimeric receptor is structurally related to the IL-10 receptor. We describe the biological activity of IL-20 in skin and show its role in psoriasis appears to be opposite to that of IL-10.

Results

Identification, Cloning, and Structural Analysis of IL-20

EST databases were searched using an algorithm designed to identify translated sequences containing both a signal sequence and one or more amphipathic helices commonly found in helical cytokines. One of the highest scores was from a single EST (INC819592) found in a human keratinocyte library (Incyte Genomics, Inc.). Based on the EST sequence, nested oligonucleotides were designed and 3′ RACE (rapid amplification of cDNA ends) was performed on RNA isolated from human skin and trachea, yielding the remainder of the coding sequence, 3′ untranslated region (UTR) and polyadenylation sequence. The 3′ UTR of this cDNA contains seven AUUUA and one perfect UUAUUUAUU mRNA instability motifs (Zubiaga et al., 1995) often found in cytokine mRNAs, which may explain its rarity in EST databases. Northern analysis indicates that this mRNA is expressed at very low levels in skin, trachea and in other tissues (data not shown).

Analysis of the entire coding sequence not only supports the classification of this protein as a helical cytokine, but also indicates that it is a member of the IL-10 family. This novel cytokine was named interleukin 20 (IL-20). Figure 1A shows a sequence comparison between human IL-20 and its three closest relatives based on amino acid sequence identity: IL-19 (40%), MDA-7 (33%), and IL-10 (28%). IL-20 also shares sequence identity with two other IL-10 family members, IL-TIF (25%) and AK-155 (24%). Mouse IL-20 cDNA was isolated from a mouse skin library using a human IL-20 coding region probe. Both human and mouse IL-20 contain 176 amino acids and are 76% identical in amino acid sequence.

[7]To whom correspondence should be addressed (e-mail: chandray@zgi.com).
[8]These authors contributed equally to this article.

A

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IL-20   MKASSLAFSLLSAAFYLLWTPSTGLKTLNLGSCVIATNLQE-----IRNG 45
IL-19   MKLQCVSLWLLGTILILCSVDNHGLR-----RCLISTDMHH-----IEES 40
MDA-7   MQMVVLPCLGFTLLLWSQVSGAQGQE-FHFGPCQVKGVVPQK----LWEA 45
IL-10   MHSSALLCCLV-LLTGVRASPGQGTQ--SENSCTHFPGNLPNMLRDLRDA 47
        *:   :    .                *  .       *          : .

IL-20   FSEIRGSVQAKDGNIDIRILRRTESLQDTKPANRCCLLRHLLRLYLDRVF 95
IL-19   FQEIKRAIQAKDTFPNVTILSTLETLQIIKPLDVCCVTKNLLAFYVDRVF 90
MDA-7   FWAVKDTNQAQDNITSARLLQQ-EVLQNVSDAESCYLVHTLLEFYLKTVF 94
IL-10   FSRVKTFFQMKDQLDNLLLKES--LLEDFKGYLGCQALSEMIQFYLEEVM 95
        *  ::  .* :*  .  :        *:  .     *      :: :*:. *:

IL-20   KNYQ--TPDHYTLRKISSLANSFLTIKKDLRLCHAHMTCHCGEEAMKKYS 143
IL-19   KDHQ--EPNPKILRKISSIANSFLYMQKTLRQCQEQRQCHCRQEATNATR 138
MDA-7   KNYHNRTVEVRTLKSFSTLANNFVLIVSQLQPSQENEMFSIRDSAHRRFL 144
IL-10   PQAE--NQDPDIKAHVNSLGENLKTLRLRLRRCHRFLPCEN--KS-KAVE 140
          .    :       ..::.:.:  :   *  .:          .: .

IL-20   QILSHFEKLEPQAAVVKALGELDILLQWMEETE------ 176
IL-19   VIHDNYDQLEVHAAAIKSLGELDVFLAWINKNHEVMSSA 177
MDA-7   LFRRAFKQLDVEAALTKALGEVDILLTWMQKFYKL---- 179
IL-10   QVKNAFNKLQ-EKGIYKAMSEFDIFINYIEAYMTMKIRN 178
          .   : :*:  .  .  *::.*..::: ::
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B

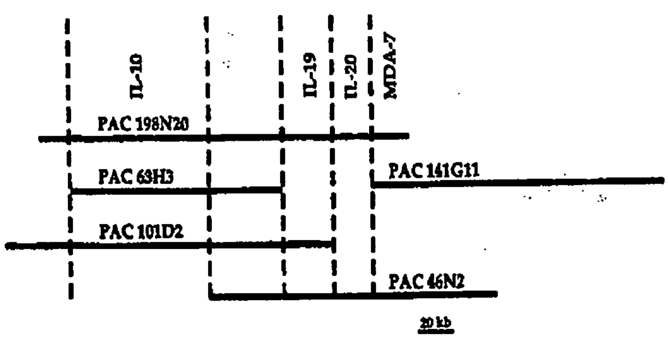

Figure 1. Multiple Alignment and Chromosomal Mapping of the IL-10 Protein Family

(A) Sequence of human IL-20 and its alignment with three other human members of the IL-10 protein family. Asterisks denote conserved residues, colons represent highly conserved residues, dots represent conservative substitutions, the arrow indicates the experimentally determined signal sequence cleavage site of IL-20, and underlines show the helical regions of IL-10. The location of conserved cysteines is indicated in bold type. (B) Chromosomal mapping of IL-20. IL-20 maps to human chromosome 1q32 and is tightly linked to IL-10, IL-19 and MDA-7.

IL-20 and IL-19 contain six conserved cysteine residues in their mature protein sequence, in contrast to IL-10, which only has four. Protein crystallographic analysis of IL-10 indicates that it forms an intercalating dimer (Zdanov et al., 1995). Threading the IL-20 sequence onto the human IL-10 structure yielded predictions about the cysteine pairing and oligomerization of IL-20. By analogy with the two disulfide bonds in IL-10, the pairings Cys33-Cys126, and Cys80-Cys132 in IL-20 can be inferred. However, residues Gln81 and Asn134 in IL-10, corresponding to Cys81 and Cys134 in IL-20, have a separation of 11.6 Å. IL-10, which forms an intercalating dimer, contains Asn116 in a potential flexible hinge region between the core of one monomer and the helix contributed by the other monomer. If Cys81 and Cys134 in IL-20 were disulfide bonded, the hinge region would be brought into close contact with the amino-terminus of helix B, leading to the prediction that IL-20 does not form an intercalating dimer. Analysis of recombinant human IL-20 produced in BHK and in baculovirus cells supports this prediction.

IL-20 Maps to a Cytokine Cluster

Radiation hybrid mapping was used to map IL-20 to human chromosome 1q32. Chromosomal localization of mouse IL-20 showed that it maps to the syntenic region on mouse chromosome 1. Since IL-10 maps to the same region (Eskdale et al., 1997), we mapped the other four IL-10 family members. MDA-7 and IL-19 also map to chromosome 1q32 (Figure 1B) whereas AK-155 and IL-TIF do not (Knappe et al., 2000 and data not shown). More detailed analysis using P1 artificial chromosome (PAC) clones allowed us to deduce the gene order on chromosome 1 as *IL-10, IL-19, IL-20, MDA-7* and to localize these genes within a 195 kb region (Figure 1B). These mapping studies indicate that four members of the IL-10 family comprise a cytokine cluster.

IL-20 Transgenic Mice Exhibit Aberrant Keratin Expression in Skin

Both human and mouse IL-20 were overexpressed in TG mice using a variety of promoters. The liver-specific mouse albumin (ALB) promoter (Pinkert et al., 1987), directing expression of human IL-20, was used initially in an attempt to achieve circulating levels of protein. Subsequent studies were conducted using the keratin 14 (K14) promoter (Vassar and Fuchs, 1989), which primarily targets expression to the epithelium; the mouse metallothionein-1 (MT-1) promoter (Palmiter et al., 1993), which gives a broad expression pattern; and the EμLCK

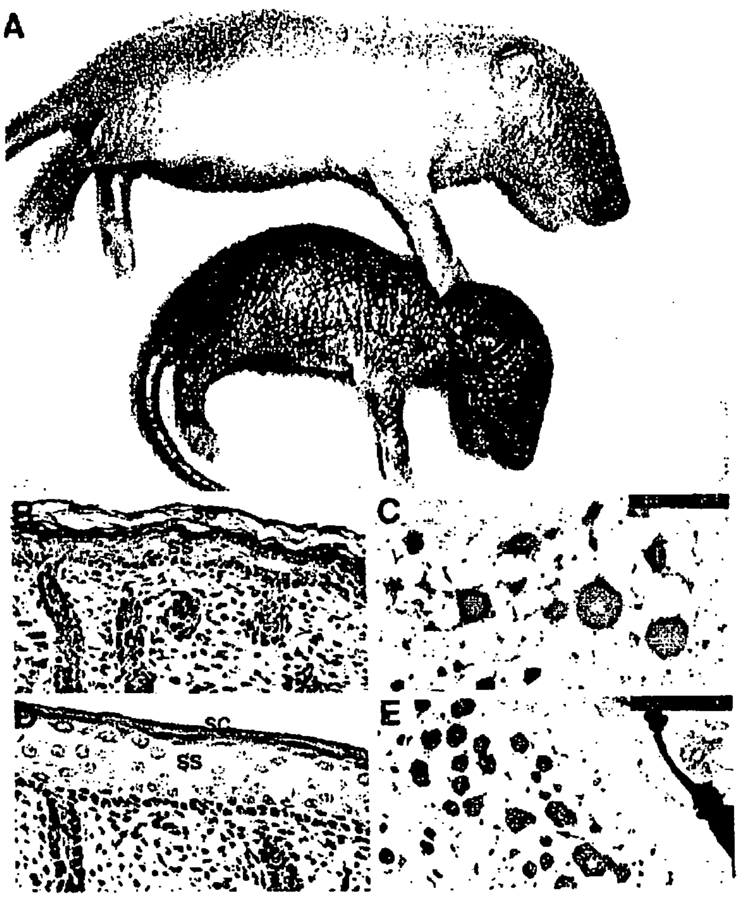

Figure 2. Skin Phenotype in IL-20 Transgenic Mice

(A) Shiny, wrinkled skin and smaller size in the IL-20 TG pup (bottom) compared to age-matched NTG littermate (top). (B) H&E-stained skin section (40×) from a 1-day-old NTG control mouse. SC = stratum corneum, SS = stratum spinosum. (C) Electron micrograph (EM) of the skin from a 1-day-old NTG mouse (16,000×). Note that the keratohyaline granules (white asterisk) are electron-dense. (D) H&E-stained skin section from a 1-day-old TG mouse with the ALB promoter driving mouse IL-20 (40×). The SC is more compact and the SS is thicker than in the age-matched control. (E) EM from a 1-day-old TG mouse with the ALB promoter driving human IL-20 (16,000×). The keratohyaline granules (white asterisk) are moth-eaten and scattered mitochondria (arrow) contain lipoid inclusions.

promoter (Iritani et al., 1997), which drives expression in cells of the lymphoid lineage. Similar results were obtained in all four cases, possibly because these promoters all give rise to circulating IL-20.

In all cases, TG pups expressing the IL-20 transgene were smaller than non-TG (NTG) littermates, had a shiny appearance with tight, wrinkled skin, and died within the first few days after birth (Figure 2A). Pups had milk in their stomachs indicating that they were able to suckle. These mice had swollen extremities, tail, nostril, and mouth regions and had difficulty moving. In addition, the mice were frail, lacked visible adipose tissue and had delayed ear and toe development. Low expression levels in liver (less than 100 mRNA molecules/cell) were sufficient for both the neonatal lethality and skin abnormalities. TG mice without a visible phenotype either did not express the transgene, did not express it at detectable levels, or were mosaic.

Histologic analysis of the skin of the IL-20 TG mice showed a thickened epidermis, hyperkeratosis and a compact stratum corneum compared to NTG littermates (Figures 2B and 2D). Serocellular crusts (scabs) were observed occasionally. Electron microscopic (EM) analysis of skin from TG mice showed intramitochondrial lipoid inclusions, mottled keratohyaline granules, and relatively few tonofilaments (Figures 2C and 2E) similar to that observed in human psoriatic skin (Gijbels et al., 1995). However, immune infiltrates commonly found in human psoriatic skin were not observed in the IL-20 TG mouse skin. In addition, many of the TG mice had apoptotic thymic lymphocytes (data not shown). No other abnormalities were detected by histopathological analysis. These histological and EM results support and extend the observed gross skin alterations.

Immunohistochemical analysis of epidermal markers was utilized to determine whether normal skin differentiation had taken place in the TG mice. These studies were performed on TG mice expressing IL-20 from the K14 promoter. Positive staining for K5 and K14 was detected in the suprabasal layer of the epidermis in addition to the expected basal layer, while staining for the hyperproliferation marker K6 in the suprabasal layer was detected in TG but not in NTG littermate skin (Figure 3). In contrast, the staining pattern for K1 (suprabasal), filaggrin (granular), and loricrin (upper spinous, granular) did not differ in the two groups of mice (Figure 3 and data not shown). Similar immunohistochemical results were obtained in TG mice expressing IL-20 from the ALB promoter (data not shown). Therefore, overexpression of IL-20 in TG mice results in aberrant expression of several keratins indicative of altered epidermal differentiation.

IL-20 TG mouse skin has a thickened epidermis and expresses K6, both suggestive of hyperproliferation. To assay directly for hyperproliferation, PCNA immunohistochemical staining was performed on skin from TG mice expressing IL-20 from the ALB and K14 promoters,

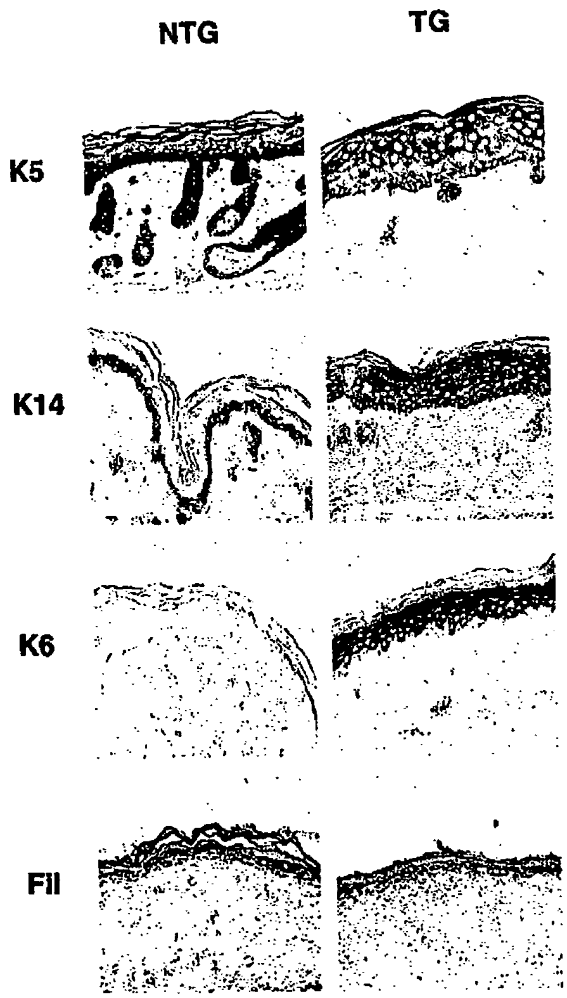

Figure 3. Aberrant Expression of Keratin Proteins Demonstrated by Immunohistochemical Analysis

K5 and K14 were detected in the basal layer of the epidermis in NTG mice. Similar sections obtained from TG mice derived from mouse IL-20 expressed from the K14 promoter show positive staining in the suprabasal layer of the epidermis as well. Also shown is positive staining for K6 in the suprabasal layer in TG mouse skin but not in NTG mouse skin. Filaggrin (Fil) staining was similar for both TG and NTG mouse skin (20×).

and from their NTG littermates. Staining in the basal skin layer was observed in NTG mice as expected, since this is the skin layer where proliferation normally takes place. However, in TG mouse skin, PCNA staining was observed in both the basal and suprabasal layers of the epidermis (data not shown). We conclude that hyperproliferation occurs in the skin of IL-20 TG mice.

Identification of a Heterodimeric IL-20 Receptor

Because IL-20 is homologous to IL-10, we hypothesized that the IL-20 receptor might share structural similarity with the heterodimeric IL-10 receptor. Therefore, we tested eight different class II cytokine receptors alone or in pairwise combinations in an IL-20 binding assay: IL-10Rα, IL-10Rβ (CRF2-4), interferon-αR1, interferon-αR2, interferon-γR1, and three orphan receptors zcytor7, zcytor11 (reported to be an IL-TIF receptor subunit; Xie et al., 2000), and DIRS1 (Parham et al., 1999). Candidate class II receptors were selected based on their expression in the human keratinocyte cell line HaCaT, previously determined to be responsive to IL-20. These receptors were transiently expressed in COS7 cells, which were then assayed for their ability to bind biotin-labeled IL-20 protein. None of the single receptor transfectants had detectable IL-20 binding. Only one pairwise combination of receptor subunits, zcytor7 (designated IL-20Rα) and DIRS1 (designated IL-20Rβ), gave rise to transfectants with positive IL-20 binding. Therefore, these proteins comprise a heterodimeric IL-20 receptor, structurally similar to the IL-10 receptor.

The specificity and affinity of IL-20 for its receptor was determined using BHK cells stably transfected with IL-20Rα, IL-20Rβ or both receptor subunits. Binding assays using radiolabeled ligand demonstrated that IL-20 bound to BHK transfectants expressing both IL-20Rα and IL-20Rβ, but not to untransfected cells nor to transfectants expressing either receptor subunit alone (Figure 4A). Binding of ^{125}I-labeled IL-20 was eliminated in the presence of 100-fold excess of unlabeled IL-20 but not in the presence of 100-fold excess of the unrelated cytokine, IL-21 (HUGO symbol for zalpha11 ligand; Novak et al., 2000). The binding data revealed 88,000 IL-20 receptors per cell with a binding affinity (K_d) of ~1.5 nM (Figure 4B).

IL-20 Binding Activates STAT3 in the HaCaT Keratinocyte Cell Line

As shown above, IL-20 binds cell lines transfected with both subunits of its receptor. However, these cell lines overexpress the IL-20 receptor relative to its normal level and their relevance to the physiological role of IL-20 is unclear. The human HaCaT keratinocyte cell line, which expresses endogenous IL-20Rα and IL-20Rβ, was used to examine IL-20 signal transduction in a biologically relevant cell type. HaCaT cells were infected with recombinant adenovirus containing a reporter construct to allow detection of intracellular signaling. The construct consists of the firefly luciferase gene driven by promoter/enhancer sequences comprised of the serum response element (SRE) and signal transducers and activators of transduction elements (STATs). This assay system detects productive ligand-receptor interactions and indicates possible downstream signal transduction components involved in receptor activation. Treatment with IL-20 alone resulted in a dose-dependent increase in luciferase activity with a half-maximal response occurring at ~2.3 nM (Figure 5A). Subsequent luciferase reporter assays using adenovirus vectors containing only the SRE element or only the STAT elements produced detectable reporter activation only through STATs (data not shown).

To determine if other cytokines act in concert with IL-20, HaCaT cells were treated with IL-20 alone or in combination with a single submaximal dose of EGF, IL-1β, or TNFα. These three proteins were chosen based on a screen of twelve cytokines and growth factors for activation of luciferase reporter constructs. Robust re-

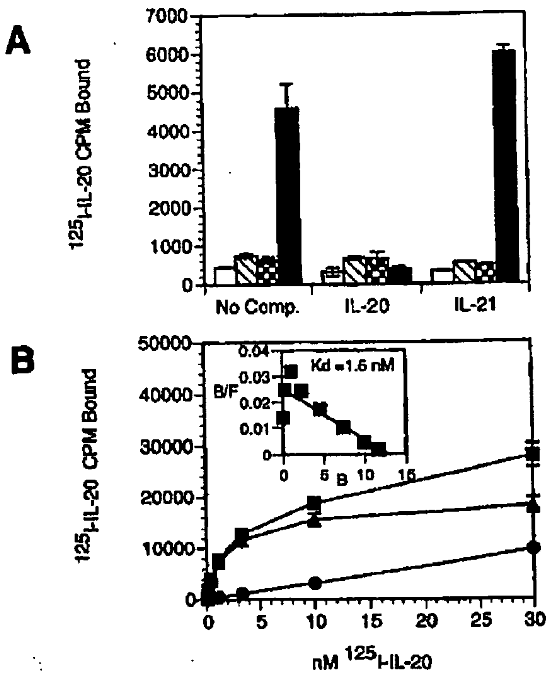

Figure 4. IL-20 Binds to the IL-20 Heterodimeric Receptor

(A) ^{125}I-IL-20 binds to transfected BHK cells expressing IL-20Rα and IL−20Rβ (solid bars), and is blocked by a 100-fold excess of IL-20 but not by IL-21. In contrast, ^{125}I-IL-20 does not bind to non-transfected BHK cells (open bars), to transfected BHK cells expressing IL-20Rβ alone (cross-hatched bars) or to IL-20Rα alone (tiled bars). (B) Saturation curve for binding of ^{125}I-IL-20 to transfected BHK cells expressing IL-20Rα and IL-20Rβ. The curves represent total binding (solid squares); nonspecific binding in the presence of 100-fold excess unlabeled IL-20 (solid circles); and specific binding determined by subtraction of nonspecific from total binding (solid triangles). A Scatchard transformation of the specific binding data is shown in the insert.

sponses were observed with EGF, IL-1β, TNF-α, and TGF-α, while KGF gave a response similar in magnitude to IL-20. The other seven cytokines, including IL-10, were inactive in this assay. In the presence of EGF, IL-1β, or TNF-α, IL-20 treatment resulted in a dose-dependent increase in luciferase activity (Figure 5A and data not shown). IL-20 in combination with IL-1β results in a half-maximal response at ~0.5 nM, about 5-fold lower than with IL-20 alone. In addition, activation of the reporter gene is detectable at 0.1 nM IL-20, a dose that is at least 10-fold lower than the IL-20 dose required alone.

BHK cells transfected with IL-20Rα, IL-20Rβ, or both receptor subunits were used to determine whether receptor pairing was required for IL-20 stimulation of STAT-luciferase. As was the case with binding assays, only cells transfected with both receptor subunits responded to IL-20 and did so with a half-maximal response of 5.7 pM (Figure 5B). We note that the IL-20 concentration for the half-maximal response in BHK cells is 400-fold lower than that for half-maximal response in HaCaT cells. It is likely that a lower concentration of IL-20 is needed for half-maximal response in BHK cells, as compared to HaCaT cells, due to higher receptor levels in the BHK IL-20 receptor transfectants.

It is well established that ligand binding to class II cytokine receptors, such as IL-10R, leads to activation of JAK kinases, phosphorylation of STATs and their subsequent translocation into the nucleus, where they activate transcription of target genes. A nuclear translocation assay was used to identify STAT proteins involved in IL-20 action. Both HaCaT cells, with endogenous IL-20 receptors, and BHK cells transfected with IL-20Rα and IL-20Rβ were treated with IL-20 protein, and translocation of STAT3 and STAT1 transcription factors from the cytoplasm to the nucleus was assayed by immunofluorescence.

In unstimulated HaCaT cells, STAT3 staining was predominantly in the cytosol (Figure 5C). Treatment of HaCaT cells with IL-20 resulted in a distinct accumulation of STAT3 in the nucleus (Figure 5D). In contrast to STAT3 translocation, HaCaT cells treated with IL-20 did not show any detectable nuclear accumulation of STAT1 (data not shown).

BHK cells transfected with IL-20Rα and IL-20Rβ were used to confirm that the IL-20 receptor was required for IL-20 stimulation of STAT3 nuclear translocation. In BHK cells lacking the IL-20 receptor, STAT3 remained cytosolic following treatment with IL-20 (Figure 5E). In contrast, in BHK cells transfected with the IL-20 receptor, STAT3 translocated to the nucleus in response to IL-20 (Figure 5F). Again, STAT1 remained cytosolic regardless of IL-20 treatment or IL-20 receptor expression (data not shown). We conclude that the IL-20 receptor is required for IL-20-mediated STAT3 activation.

To screen for potential downstream target genes for IL-20 activity, microarray analysis was performed on mRNA isolated from HaCaT cells treated with IL-20, IL-1, or IL-20 plus IL-1. Human cytokine, growth factor, and receptor genes were represented on the microarray. Expression of a number of genes increased 2- to 4-fold in response to IL-20 alone. In addition, a different, but overlapping, set of genes had higher expression in response to IL-20 + IL-1 than the sum of the individual cytokine responses (Experimental Procedures). A high percentage of these genes are involved in inflammation. RT-PCR analysis on three of these genes, *TNF-α*, *MRP-14*, and *MCP-1*, confirmed these results (data not shown). We conclude that the magnitude of specific target gene expression based on microarray and RT-PCR results correlated with those of reporter gene activation in HaCaT cells. IL-20 alone exhibited a modest increase in specific target gene expression and showed enhancement of the IL-1-induced expression of at least three genes known to be involved in inflammatory responses.

Expression of IL-20Rα and IL-20Rβ in Skin and Upregulation in Psoriasis

The expression pattern of IL-20Rα and IL-20Rβ in a variety of human tissues was determined by RT-PCR analysis. Both IL-20Rα and IL-20Rβ are most highly expressed in normal skin and testis, with lower expression in a variety of other normal tissues (Figure 6). In addition, IL-20Rα mRNA appears to be expressed in many tissue types where IL-20Rβ expression is undetectable under similar conditions. Most significant for the present study

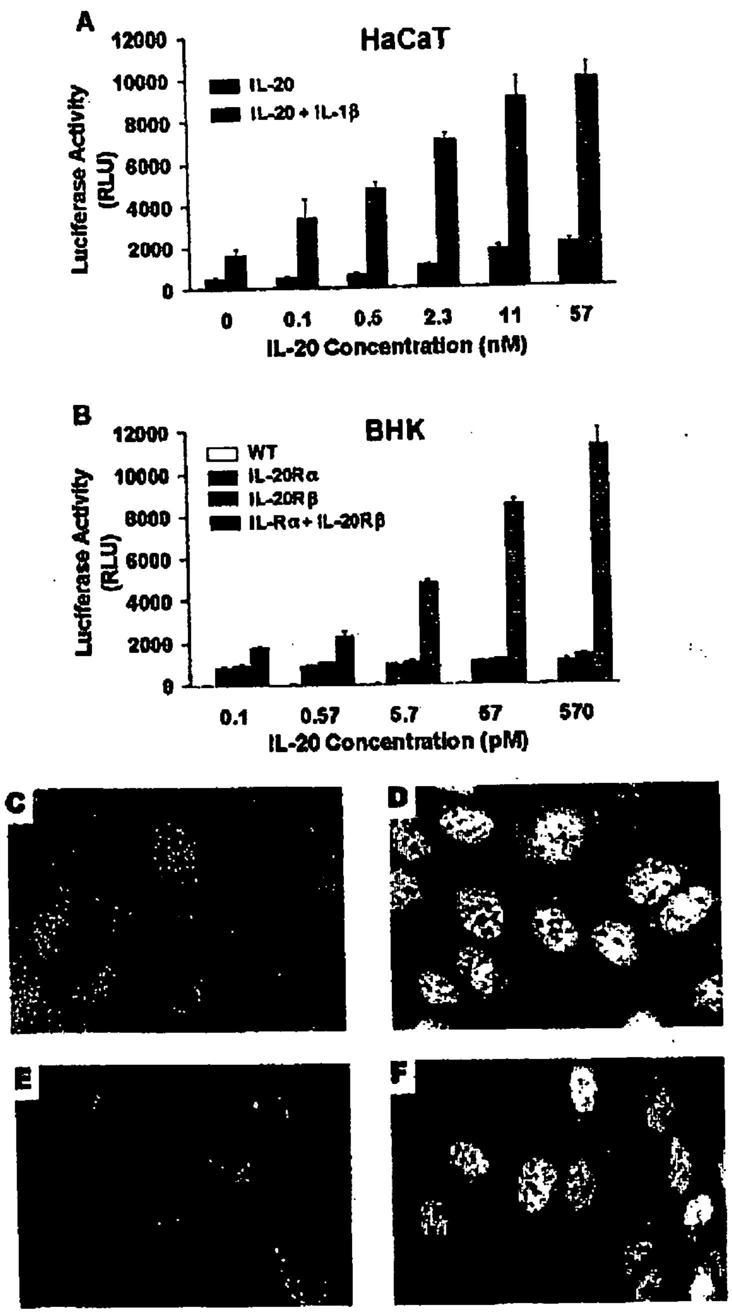

Figure 5. IL-20 Stimulates a Signal Transduction Pathway through STAT3

Panels (A) and (B) utilize luciferase reporter constructs to detect intracellular signaling. (A) HaCaT cells were infected with an adenovirus construct containing SRE and STAT promoter elements driving expression of luciferase. Treatment of HaCaT cells with IL-20 alone results in a dose-dependent increase in luciferase activity, which is dramatically enhanced by the addition of 0.1 nM IL-1β. (B) BHK cells were stably transfected with STAT promoter elements driving expression of luciferase. Treatment of BHK cells expressing IL-20Rα and IL-20Rβ with IL-20 results in a dose-dependent increase in luciferase activity. Panels (C)–(F) assay the translocation of STAT3 transcription factor from the cytoplasm to the nucleus by immunofluorescence. STAT3 staining was predominantly in the cytosol in unstimulated HaCaT cells (C), whereas treatment of HaCaT cells with IL-20 resulted in a distinct nuclear accumulation of STAT3 (D). BHK cells lacking the IL-20 receptor had cytosolic STAT3 following treatment with IL-20 (E), whereas BHK cells transfected with IL-20Rα and IL-20Rβ, had STAT3 translocation to the nucleus in response to IL-20 (F).

is that IL-20Rα and IL-20Rβ are both expressed in skin, where they are involved in IL-20 binding and activation of signal transduction.

We hypothesized that the IL-20 receptor may have altered regulation in skin diseases. To test this hypothesis, in situ hybridization was performed on skin samples from seven psoriasis patients and three patients with normal skin. All seven psoriatic skin samples had high levels of IL-20Rα and IL-20Rβ mRNA in keratinocytes whereas normal skin samples had minimal to undetectable levels of either receptor subunit mRNA (Figures 7A–7F). The difference between these in situ hybridization results and the RT-PCR results described above for normal skin may reflect the higher sensitivity of the RT-PCR assay. In some of the psoriatic skin samples, positive signals were also observed for both IL-20 receptor subunits in mononuclear immune cells and in endothelial cells in a subset of vessels (Figures 7G and 7H). Therefore, both IL-20Rα and IL-20Rβ are expressed in keratinocytes, immune cells, and endothelial cells, the

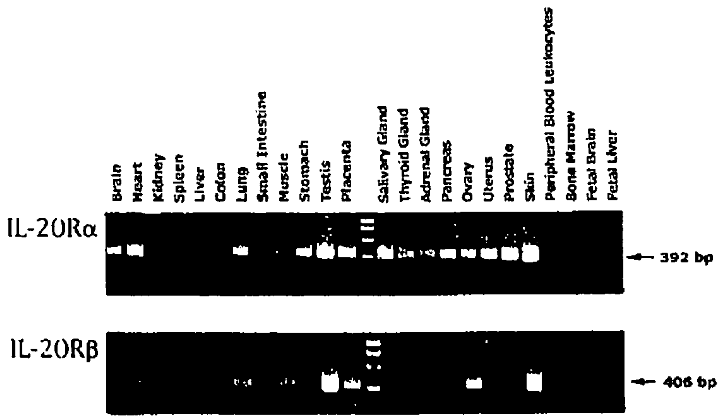

Figure 6. Expression of IL-20Rα and IL-20Rβ in Human Tissues

RT-PCR analysis of IL-20Rα and IL-20Rβ was performed using a panel of 24 human tissues. Both receptor subunit mRNAs are highly expressed in skin and testis.

major cell types thought to interact in psoriasis (Bos and De Rie, 1999; Karasek, 1999).

Discussion

IL-20 stimulates signal transduction in the human keratinocyte HaCaT cell line, supporting a direct action of this novel ligand in skin. In addition, IL-1β, EGF, and TNF-α, proteins known to be active in keratinocytes and to be involved with proliferative and proinflammatory signals in skin, enhance the response to IL-20. IL-20 may be a cytokine that exhibits its most potent effects in combination with other factors, similar to the role that stem cell factor and flt-3 ligand play in the hematopoietic system (Koller et al., 1996). In both HaCaT and BHK cells expressing the IL-20 receptor, IL-20 signals through STAT3 as does IL-10 (Kotenko and Pestka, 2000). IL-10 also activates STAT1 to some extent, which was not observed for IL-20. Future experiments will be directed at defining additional components of the IL-20 signal transduction pathway and at understanding the role of its inflammation-related target genes.

Colocalization of IL-20 with three other IL-10 family members identifies the 1q32 region as a cluster of cytokine genes. Clustering of family members may be the result of gene duplication with subsequent divergence of function and regulation. The known activities for the IL-10 family members in this cluster reflect this functional divergence. IL-10 has anti-inflammatory activity in skin due to inhibition of proinflammatory cytokine synthesis. Consistent with this observation, expression of both IL-10 and the IL-10 receptor are decreased in psoriatic skin (Michel et al., 1997; Asadullah et al., 1998). In contrast, expression of both IL-20 receptor subunits is increased in psoriatic skin. MDA-7 expression is elevated in melanoma cells and is thought to have antitumor activity (Jiang et al., 1996), while the actions of IL-19 have not been described. Further experimentation is needed to elucidate the functions of the new members of this IL-10 family to more comprehensively understand their similarities and differences.

Two orphan class II cytokine receptors, both of which are expressed in skin, were identified as IL-20 receptor subunits. Both IL-20 receptor subunits are required for ligand binding, distinguishing their role from that of subunits in the four other known class II cytokine receptors (Domanski and Colamonici, 1996; Pestka et al., 1997; Xie et al., 2000). For example, in the IL-10 heterodimeric receptor, the IL-10Rα subunit is sufficient for high-affinity ligand binding, while IL-10Rβ is required for signaling (Kotenko et al., 1997). IL-20Rα and IL-20Rβ are also coexpressed in a number of human tissues besides skin, suggesting additional target tissues for IL-20 action. Additionally, we have detected IL-20Rα mRNA, but not IL-20Rβ mRNA, in several human tissues suggesting that IL-20Rα may partner with other class II receptor subunits. However, unlike the class I cytokine receptor common signaling subunits β_c, gp130, and IL2Rγ, there is only one example of a shared receptor subunit (CRF2-4) in the class II receptor family (Dumoutier et al., 2000b; Xie et al., 2000). It is also possible that the IL20Rα/IL20Rβ heterodimeric receptor acts as a receptor for other IL-10 family members. We conclude that the IL-20 heterodimeric receptor is structurally similar to other class II cytokine receptors and is expressed in skin where we have demonstrated activity of the IL-20 ligand.

Two lines of evidence suggest a role for IL-20 and its receptor in psoriasis. This multigenic skin disease is characterized by increased keratinocyte proliferation, altered keratinocyte differentiation, and infiltration of immune cells into the skin (Bos and De Rie, 1999). The first line of evidence for a role of IL-20 in psoriasis is that the observed hyperkeratosis, thickened epidermis, and proliferation in the suprabasal layer in the TG mice that resemble human psoriatic abnormalities. Changes detected in the epidermal protein expression in IL-20 TG mice include the presence of the basal K5 and K14 in both the basal and suprabasal layers, similar to the changes observed in human psoriatic skin (Sun et al., 1985; Castelijns et al., 1999). IL-20 TG mice also express the hyperproliferative-associated K6, present in human psoriatic but not normal skin. Similar skin abnormalities have been reported in TG mouse models expressing KGF, TGF-α, and interferon γ from epidermal-specific promoters (Vassar and Fuchs, 1991; Guo et al., 1993; Carroll et al., 1997). No TG mouse model demonstrating all aspects of human psoriasis exists. For example, immune infiltrates were not observed in the IL-20 TG mice. In addition, the ultrastructural features of the IL-20 TG

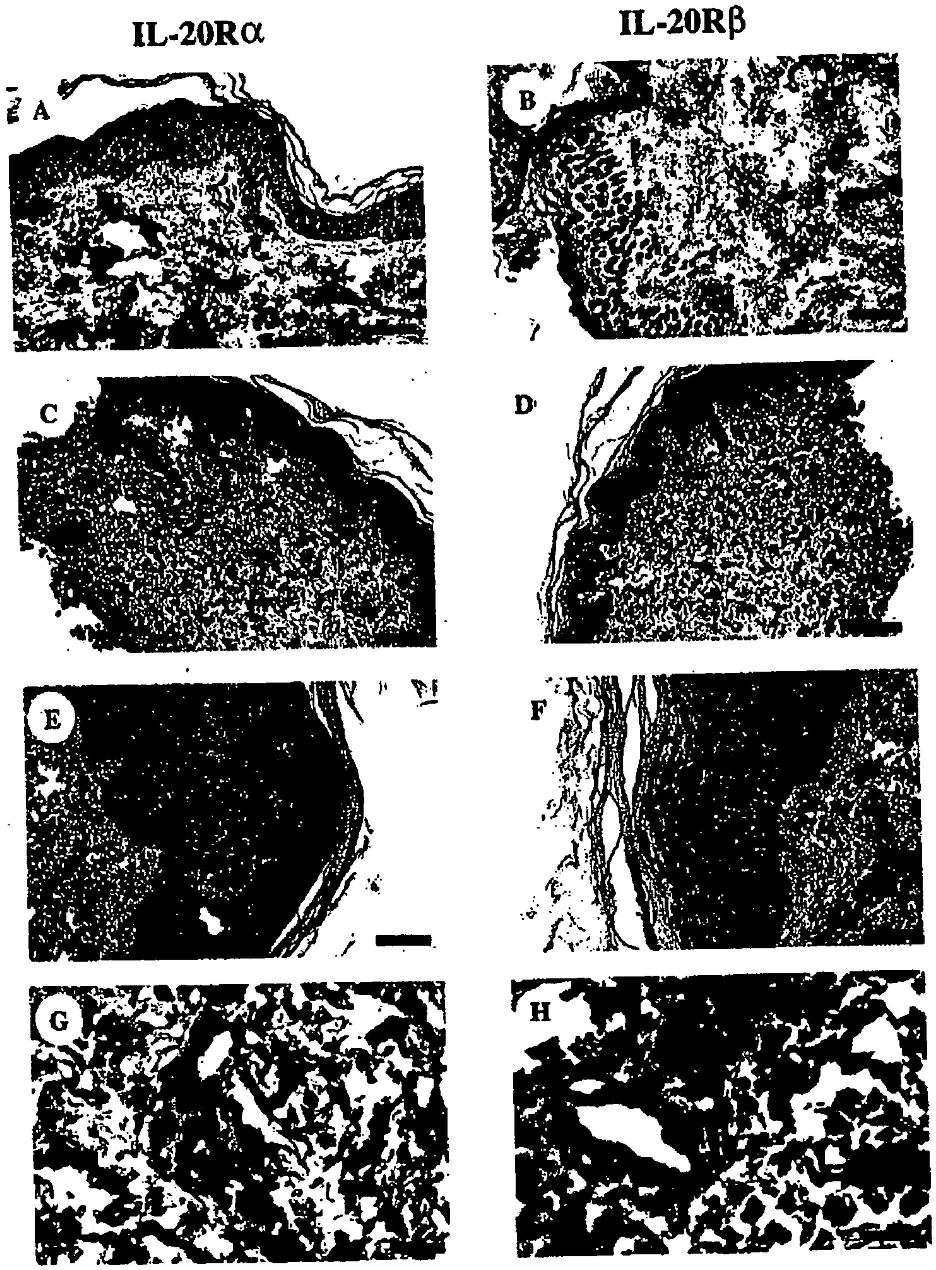

Figure 7. In Situ Hybridization Demonstrates IL-20Rα and IL-20Rβ mRNA Upregulation in Psoriasis
IL-20Rα mRNA expression is shown in (A), (C), (E), and (G), whereas IL-20Rβ mRNA expression is shown in (B), (D), (F), and (H). Normal human skin shows minimal to undetectable levels of both IL-20Rα and IL-20Rβ mRNA (A and B). In contrast, increased staining for both receptor subunits is observed in human psoriatic skin (C–H). Upregulation of both receptor subunit mRNAs is detected in keratinocytes (E and F), as well as in endothelial and immune cells (G and H). Scale bar indicates: (A and B) = 1 mm; (C and D) = 2 mm; (E and F) = 0.6 mm; and (G and H) = 0.2 mm.

mouse skin have been found in other hyperproliferative diseases of the epidermis. Decreased numbers of tonofilaments, thought to be related to defective keratinization, are a striking feature of human psoriasis (Jahn et al., 1988). Intramitochondrial inclusions have been found in both chemically induced and naturally occurring hyperplastic skin conditions in mice (Gijbels et al., 1995). Finally, mottled keratohyaline granules were found in neonatal TGFα TG mice that had thickened skin due to epidermal proliferation (Vassar and Fuchs, 1991). We conclude that IL-20 TG mice appear to exhibit many of the characteristics observed in human psoriasis.

A second line of evidence that implicates the IL-20 receptor in psoriasis is that both IL-20Rα and IL-20Rβ mRNA are markedly upregulated in human psoriatic skin compared to normal skin. Both IL-20 receptor subunits are expressed in keratinocytes throughout the epidermis and are also expressed in a subset of immune and endothelial cells. We propose that increased expression of an activated IL-20 receptor may alter the interactions between endothelial cells, immune cells and keratinocytes, leading to dysregulation of keratinocyte proliferation and differentiation.

A crucial step in understanding the function of a novel cytokine is the identification and characterization of its cognate receptor. We have successfully used a structure-based approach to isolate a novel interleukin and defined skin as a target tissue, which ultimately led to the isolation of its receptor. We demonstrate that IL-20 binds its receptor on keratinocytes and stimulates a STAT3-containing signal transduction pathway. The effect of IL-20 on skin differentiation in TG mice, together with the upregulation of the IL-20 receptor subunits in psoriasis, pave the way for future studies on this ligand/receptor pair in inflammatory skin diseases.

Experimental Procedures

Bioinformatics Analysis

A novel "quasi-threading" algorithm, which inspects sequences for multiple profiles, was employed. Ungapped amino acid profiles of width n are two-dimensional matrices with dimensions n by 20 (Gribskov et al., 1987). Profiles are used to provide the score for amino acids in each position of a polypeptide segment of length n. Translated EST and other DNA database sequences were threaded using a protein structure-based profile linked by regions of variable length. Two profiles of width 15, which described a signal sequence and an amino-terminal amphipathic helix typical of helical cytokines with a variable region from 1 to 30 residues, were used.

Cloning of Mouse IL-20

Using a probe corresponding to the coding region of human IL-20, a mouse genomic lambda library (Clontech) was screened at low stringency, potential positives were identified and plaque purified to homogeneity, and subclones were generated for sequencing. The sequence of one subclone revealed two predicted exons homologous to human IL-20. Oligonucleotides were designed to this sequence and used for 5' and 3' RACE on mouse skin Marathon-ready cDNA, to generate the full-length coding sequence for mouse IL-20.

Chromosomal Mapping of IL-20

IL-20 was mapped to chromosome 1 on both the lower resolution GeneBridge 4 and the medium resolution Stanford G3 radiation hybrid (RH) mapping panels. Mouse IL-20 was mapped by PCR using the commercially available mouse T31 whole genome radiation hybrid (WGRH) panel (Research Genetics, Inc.) and IL-20 murine-specific sense and anti-sense primers. IL-10, IL-19, and MDA-7 were mapped by PCR using the commercially available version of the Stanford G3 Human/Hamster RH panel (Research Genetics, Inc.).

A PAC library and high-density array filters were obtained from Roswell Park Cancer Institute and were hybridized with probes from the coding regions of human IL-19 and MDA-7. Four PAC clones were identified in this screen. Two additional clones (63B3 and 63H3) were identified in a library from Genome Systems, using a PCR screen with IL-10 primers. Inserts were sized using NotI digestion to excise inserts, followed by pulsed-field gel electrophoresis. To determine gene order, PACs were analyzed using PCR (IL-19, MDA-7, and IL10) and hybridization (IL-20, MDA-7). PAC 141G11 was negative by PCR for MDA-7, but was positive by hybridization, indicating that the PAC contains part of the gene but is missing the MDA-7 primer region.

IL-20 Transgenic Mice

Both human and mouse IL-20 cDNA coding sequences were amplified by PCR and cloned into plasmids containing different promoters. Introns from rat insulin II, SV40 T-antigen, or human growth hormone were cloned either 5' or 3' of the IL-20 cDNA to improve expression. Primers contained a consensus translation initiation site upstream of the ATG. All plasmids used contain a 650 bp human growth hormone (hGH) poly A sequence 3' of the coding sequence.

One half centimeter tail snips were isolated from pups (C57/Bl6 X C3H F2 hybrid mice) resulting from microinjection of the linearized expression cassette DNA. Genomic DNA was isolated using the DNAeasy kit (Qiagen). Genotyping was performed by PCR using three sets of primers against specific regions of the TG plasmids: hGH 3' UTR, flanking regions for the IL-20 cDNA insert, and an endogenous mouse gene.

Mouse tissues were dissected, stored in RNAlater (Ambion) and total RNA was isolated using the RNAeasy kit (Qiagen). Expression analysis of IL-20 TG mRNA was performed using the Taqman RT-PCR assay (Perkin Elmer) with primers against the hGH 3' UTR.

Histological Analysis

Tissues for light microscopy were collected in 10% neutral buffered formalin, routinely processed, sectioned at 5 μm and stained with hematoxylin and eosin. Skin sections for electron microscopy were collected into paraformaldehyde-glutaraldehyde, routinely processed, and stained with uranyl acetate (Sheehan and Hrapchak, 1980).

Immunohistochemistry for Epidermal Proteins

Carnoy's fixed skin from the backs of normal and TG (newborn to day 3) mice were sectioned for immunohistochemistry. Polyclonal antibodies to mouse keratins 1, 5, 6, 10, and 14 as well as polyclonal antibodies to mouse loricrin, involucrin and pro/filaggrin (Berkeley Antibody Company) were followed by secondary biotinylated anti-rabbit IgG, amplified with immunoperoxidase (Vectastain ABC kit, Vector Laboratories), and visualized with diaminobenzidine as chromagen. PCNA immunohistochemistry was performed as described by Dietrich (1993) on skin from TG mice expressing IL-20 from the ALB and K14 promoters, and from their NTG littermates.

Recombinant IL-20

IL-20 cDNA was generated by PCR and inserted into the pZP9NEE mammalian expression vector containing the MT-1 promoter or the baculovirus expression vector pZBV3L (a modification of an Invitrogen vector) containing the basic protein promoter. NEE is an N-terminal sequence tag with the sequence EYMPMEGS. The pZP9NEE-IL-20 vector was transfected into BHK570 cells by lipofectamine and selected in DMEM + 5% FBS with 1 μM methotrexate. Supernatants from resistant cells were analyzed for protein and high expressing clones were scaled up in cell factories. Protein was purified from filtered BHK570 culture supernatants by EE monoclonal antibody affinity chromatography (Grussenmeyer et al., 1985).

Receptor Binding Assays

A modification of the "secretion trap" (Davis et al., 1996) was performed on COS7 cells transfected with candidate class II cytokine receptor cDNAs.

Cell Culture and Transfections

HaCaT cells (Boukamp et al., 1988), were obtained from Dr. N. Fusenig. BHK570 cells are deposited at ATCC (CRL-10314). Stable BHK cell lines were generated by selection in puromycin (IL-20-Rα) and zeocin (IL-20-Rβ).

Binding Assays

Human IL-20 was iodinated using iodobeads (Pierce). For competition binding assays, 250 pM ^{125}I-IL-20 was used with or without 25 nM competitor proteins. For saturation binding, a 30 nM to 13.7 pM dilution series of ^{125}I-IL-20 was used, with or without a 100-fold excess of

unlabeled IL-20 for determination of specific binding. Scatchard analysis was done using Delta Graph 4.0 (Delta Point, Inc.).

Luciferase Assays

CMV promoter/enhancer and SV40 pA sequences were removed from pACCMV.pLpA (Becker et al., 1994) and replaced with a linker containing KpnI and HindIII sites. The STAT/SRE-driven luciferase reporter cassette was excised from vector KZ136 (Poulsen et al., 1998) as a Kpn-HindIII fragment and inserted into the vector described above. Recombinant KZ136 adenovirus was produced by transfection with JM17 adenovirus into 293 cells (Becker et al., 1994). Plaque purified virus was amplified and used to infect cultured cells at 5–50 pfu/cell 12–36 hr before assay. Luciferase reporter assays were performed with a 4 hr induction time following treatment (Poulsen et al., 1998).

STAT Nuclear Translocation Assay

HaCaT or BHK cells were treated with IL-20, interferon-γ (positive control for STAT1 translocation), or interferon-α (positive control for STAT3 translocation) for 20 min and analyzed using STAT1 and STAT3 HitKit™ reagent kits (Cellomics, Inc). Cells were incubated with anti-STAT3 or anti-STAT1 antibody (rabbit polyclonal) for 1 hr, followed by incubation with secondary antibody (AlexaFluor® 488 conjugated goat anti-rabbit IgG) and Hoechst 33342 dye. Cells were examined on an inverted, fluorescent microscope and images recorded with a CCD camera. To generate a dose-response curve and calculate the IL-20 concentration for a half-maximal response, fields were scanned, images were acquired and analyzed by the ArrayScan® II system (Cellomics®, Inc.) using an image processing algorithm for cytosol-nuclear translocation (Ding et al., 1998).

Microarray Assays

HaCaT cells were grown to post-confluence and were switched to serum-free media for two days. Cells were then treated as follows for 4 hr: IL-1α (5 ng/ml), IL-1α (5 ng/ml) + IL-20 (1 μg/ml), IL-20 (1 μg/ml), or no cytokine addition. Total RNA was isolated by CsCl gradient centrifugation. Protocols for polyA⁺ enrichment, probe synthesis and hybridization to Atlas™ arrays were provided by Clontech. Array membranes were exposed on a phosphorimager screen overnight and analyzed using AtlasImage™ 1.0 software (Clontech).

Genes whose expression increased at least 2-fold with IL-20 addition (average fold increase): TNF-α (2.2) and MRP-14 (3.4). Genes with enhanced expression due to the combination of IL-20 and IL-1 (IL-20 alone, IL-1 alone, IL-20 + IL-1): MRP-14 (3.0, 12.2, 20.3) and MCP-1 (1.3, 32.4, 55.9).

RT-PCR Analysis on Human Tissue

RT-PCR was performed on Rapid-Scan human gene expression panels (Origene Technologies, Inc.). Oligonucleotide primers 5' TCAAACAGAACGTGGTCCCAGTG 3' and 5' TCCGAGATATTGAGG GTGATAAAG 3' were used to generate a 392 bp IL-20Rα fragment. Oligonucleotide primers 5' GCTGGTGCTCACTCACTGAAGGT 3' and 5' TCTGTCTGGCTGAAGGCGCTGTA 3' were used to generate a 406 bp IL-20Rβ fragment. PCR conditions were 94°C for 3 min followed by 35 cycles of 94°C/30 s, 64°C/30 s, 72°C/120 s, followed by 72°C for 5 min.

In Situ Hybridization

In situ hybridization probes were designed against human IL-20Rα and IL-20Rβ. Blastn searches against our databases, which include all known class II cytokine receptors, demonstrated that the probes were specific for their respective receptors. PCR products were used as templates for synthesis of digoxygenin-labeled antisense RNA probes. Tissues were fixed in 10% buffered formalin and embedded in paraffin. Hybridization was carried out at 60°C with 50% formamide/2× SSC. The signals were amplified with two to three rounds of tyramide signal amplification (TSA *in situ* indirect kit, NEN) and visualized with Vector Red substrate kit (Vector Lab). Slides were counterstained with hematoxylin. All tissues were tested with positive control probes.

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GenBank Accession Numbers

The GenBank accession number for human IL-20 is AF224266 and for mouse IL-20 is AF224267. The human gene symbol *IL-20* was approved by the HUGO Nomenclature Committee.

IL-19 was discovered by Human Genome Sciences, Inc. and is described in the International patent application WO 98/08870. It was independently discovered at ZymoGenetics and assigned GenBank accession number AF192498.

The three orphan class II cytokine receptors are zcytor7 and zcytor11 (ZymoGenetics) and DIRS1 (Schering). The GenBank accession number for zcytor7 is AF184971 described in U.S. Patent No. 5,945,511 (1999). The GenBank accession number for zcytor11 is X24379, described in international patent application publication number WO 2000/39161. Human DIRS1 was described in international patent application publication number WO 99/46379 (1999) by Schering Corporation. The human gene symbols *IL-20RA* (encoding IL-20Rα) and *IL-20RB* (encoding IL-20Rβ) were accepted by the HUGO Nomenclature Committee.

Cytokines: **IL-20 – a new effector in skin inflammation**

Benjamin E. Rich and Thomas S. Kupper

The newly discovered cytokine interleukin-20 (IL-20) is structurally related to IL-10, yet it appears to be an autocrine factor for keratinocytes that regulates their participation in inflammation.

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As the DNA sequences of mammalian chromosomes and vast cDNA libraries become available, researchers are vigorously scouring them for their favorite genes. The search for new cytokines has been among the most competitive and indeed one of the latest finds, interleukin-20 (IL-20), was discovered *in silico*. Using a computational method to identify sequences encoding amphipathic α helices as well as a signal peptide, Blumberg *et al.* [1] focused on a high-scoring cDNA from a keratinocyte library. This cDNA was found to encode a relative of the cytokine IL-10 and was named IL-20. Biological studies of IL-20 revealed that it has an important role in promoting hyperproliferation of keratinocytes and thereby modulating inflammation in the skin.

A new member of the IL-10 family

The IL-20 mRNA contains motifs in its 3′ untranslated region that are associated with instability. In agreement with this, the IL-20 mRNA appears to be rare and short-lived. The polypeptide sequence of IL-20 is similar (20–40% identical) to that of IL-10 and four other mammalian cytokines, as well as several viral products that constitute the IL-10 family (Table 1). The presence of IL-10-related genes in various viral genomes may reflect the ability of these factors to act as autocrine growth factors for infected cells as well as regulatory signals to parry host immune responses. Further application of sensitive algorithms to the complete sequences of the human and other mammalian genomes is likely to identify any remaining members of this family (as well as other cytokine families) in the near future.

The genes encoding the known human IL-10-related cytokines are clustered in two loci. The genes for IL-10, IL-19, IL-20 and the melanocyte differentiation-associated factor MDA-7 are found within a 200 kb region of chromosome 1, whereas genes encoding the two other family members — IL-22 and AK155 (a factor induced upon transformation by Herpesvirus) — are found within 30 kb of each other and less than 100 kb from the interferon-γ (IFN-γ) gene on chromosome 12. Certain alleles of this region of chromosome 12 have been found to be associated with asthma and inflammatory bowel disease [2]. While initial attention was drawn to the IFN-γ gene, allelic variants have not been found. Therefore it remains possible that variations in either the IL-22 or AK155 genes contribute to these diseases.

Transgenic mice reveal skin as a major target of IL-20

To investigate the biology of IL-20 *in vivo*, several transgenic mice were generated that expressed IL-20 under the control of various tissue-specific promoters. All of these mice were runted at birth and died within days. Histological analysis revealed a profoundly thickened epidermis characterized by increased numbers of keratinocytes and expression in the suprabasal layers of differentiation and proliferation markers that are normally confined to the basal layer. These changes appeared to be caused by circulating IL-20, because even mice expressing the transgene in tissues away from the skin, such as in the liver (driven by the albumin promoter), were similarly affected.

In light of the effects of IL-20 on the epidermis of the transgenic mice, Blumberg *et al.* [1] focused their attention on keratinocytes. IL-20 was found to activate signal transducer and activator of transcription 3 (Stat3), one of the transcriptional activators involved in IL-10 signaling, in an immortalized keratinocyte cell line (HaCaT). Exposure of HaCaT cells to IL-20 induces translocation of Stat3 to the nucleus and stimulation of transcription from a Stat3-responsive reporter gene. Stat3 can be activated by more than a dozen different factors in different cell types. Its central role in development is demonstrated by the observation that Stat3-deficient mice die as early embryos. Specific deletion of the Stat3 gene in keratinocytes blocks their responses to epidermal growth factor (EGF), hepatocyte growth factor (HGF) and IL-6 [3]. Although Stat3-deficient keratinocytes can form approximately normal skin, they are defective in wound healing and have altered secondary hair cycles. While EGF is an important cytokine for keratinocytes, some of the deficits in Stat3-deficient keratinocytes may also be due to their inability to respond to IL-20, given the recent study by Blumberg *et al.* [1]. In addition, as IL-10 signal transduction includes both Stat3-dependent and -independent pathways, it is possible, by analogy, that IL-20 signal transduction is similarly complex [4].

When HaCaT cells were incubated with IL-20 in the presence of submaximal concentrations of IL-1β, tumor necrosis factor α (TNF-α) or EGF, strong cooperativity was

Table 1

The IL-10 family of cytokines.

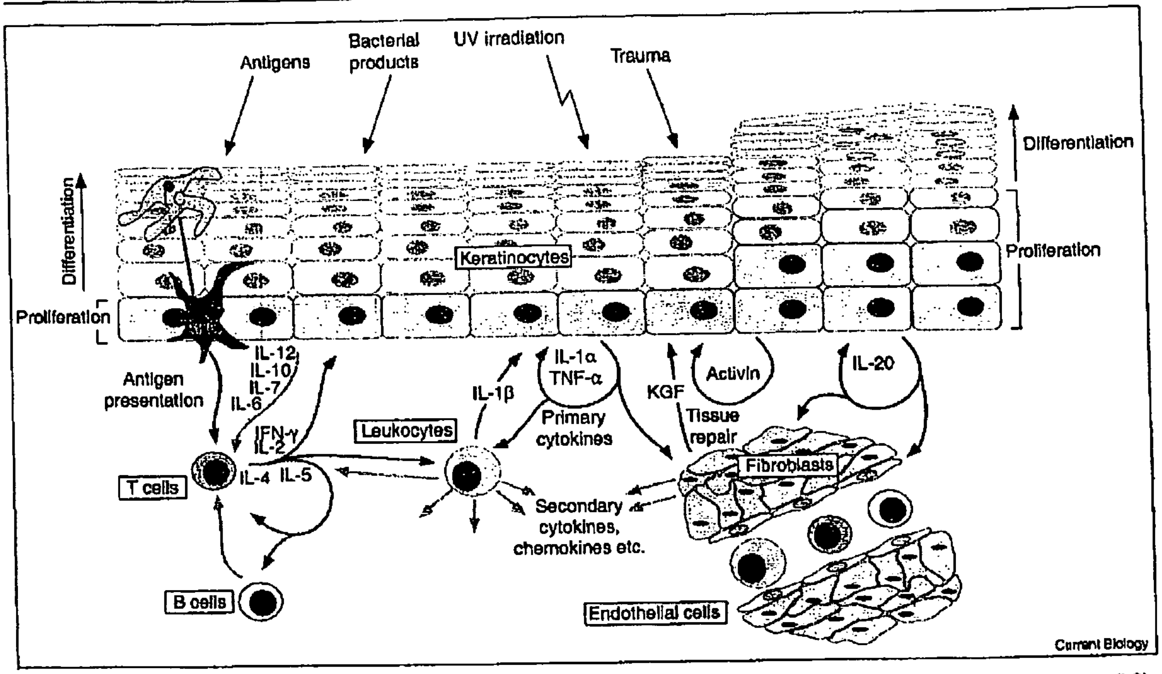
Cytokine	Gene location	Cellular source	Cognate receptor	Biological activities	References
IL-10	Human 1q32	Activated T cells, B cells, monocytes, keratinocytes	IL-10Rα, IL-10Rβ	Enhances Th2 differentiation; suppresses Th1 differentiation	[9]
IL-19	Human 1q32	Stimulated monocytes	Unknown: not IL-10Rα, IL-10Rβ	Unknown	[10]
IL-20	Human 1q32	cDNA found in a keratinocyte library	IL-20Rα, IL-20Rβ; both upregulated in psoriasis	Stimulates keratinocyte proliferation and differentiation; activates Stat3; receptor also on certain endothelial and mononuclear cells	[1]
MDA-7 (mob-5, C49a)	Human 1q32	Melanocytes, stimulated cells, melanoma cells, fibroblasts in wound repair, *ras*-transformed cells	Binding activity detected on *ras*-transformed cells	Expression associated with differentiation of melanocytes; reverts malignant phenotype of melanoma; upregulated in wound healing; autocrine factor of *ras*-transformed cells	[11–13]
IL-22 (IL-TIF)	Human 12q15	IL-9- or lectin-stimulated T cells, mast cells	IL-22Rα, IL-10Rβ	Activates Stat1, 3, 5; stimulates monocytes to make TNF	[14–16]
AK155	Human 12q15	*Herpesvirus saimiri* transformed T cells	Unknown	Transformation of T cells?	[17]
ebvIL-10 (BCRF1)	Epstein–Barr virus (EBV) genome	EBV-infected cells	IL-10Rα, IL-10Rβ	Similar to IL-10 but lower affinity; may block immune response to viral infected cells; autocrine growth factor for infected B cells.	[18,19]
cmvIL-10	Cytomegalovirus (CMV) genome	CMV-infected cells	IL-10Rα, IL-10Rβ	Similar to IL-10, may block immune response to virus-infected cells	[20]
hpvIL-10	*Herpesvirus papio* genome	Unknown	Unknown	Unknown	Genbank accession AAF23949
yldvIL-10	Yaba-like disease virus genome	Unknown	Unknown	Unknown	Genbank accession NP_073519

observed, increasing their sensitivity to IL-20 by as much as 10-fold and quadrupling the magnitude of their response. Three genes found to be upregulated by IL-20 in HaCaT cells encode proteins involved in inflammation: TNF-α, which is likely to further enhance the response of keratinocytes to IL-20 and also stimulates chemotaxis and antimicrobial activity of myeloid cells; the chemokine MCP-1, involved in leukocyte chemotaxis; and MRP-14, one of the S100 calcium-binding proteins implicated in neutrophil integrin activation and associated with epidermal inflammation [5].

The IL-20 receptor is upregulated in psoriasis

Receptor chains expressed in HaCaT cells were examined and two previously known but orphan class II cytokine receptor chains (now named IL-20Rα and IL-20Rβ) were found to form a functional cognate receptor for IL-20 when co-transfected into a different cell line. Because suprabasal expression of basal keratins and proliferation markers, as well as certain structural alterations in the epidermis of the IL-20 transgenic mice are similar to features of psoriasis, expression of the two receptor chains was measured in normal and psoriatic human skin. While very low levels were detected in normal skin, dramatic induction of both chains was found in keratinocytes, endothelial cells and certain mononuclear cells in psoriatic lesions. The hyperproliferation of keratinocytes associated with psoriasis is dependent upon the participation of activated T cells [6]; IFN-γ, normally a product of activated T cells, provokes similar hyperproliferation of keratinocytes [7]. Therefore it seems likely that expression of the IL-20 receptor, and perhaps IL-20, by keratinocytes is stimulated by IFN-γ or other products of activated T cells. Whether in concert with other factors or expressed by

Figure 1



Signaling pathways in inflammation of the skin. Basal layers of keratinocytes proliferate while upper levels differentiate to form the barrier. Changes in proliferation and differentiation alter the structure of the skin. External stimuli such as trauma or irradiation cause keratinocytes to release primary cytokines, IL-1α and TNF-α. These factors provoke fibroblasts, leukocytes and keratinocytes to express more primary cytokines (IL-1) as well as proinflammatory secondary cytokines and chemokines. Trauma also elicits KGF and activin expression which promote repair. Foreign antigens are captured by Langerhan's cells (LC) and other antigen-presenting cells (APC) which migrate and interact with T cells. Activated T cells, supported by secondary cytokines from the epidermis, dermis and leukocytes, proliferate and release cytokines that promote cellular (Th1, involving IFN-γ) or humoral (Th2, involving IL-4) immune responses. Signals that control IL-20 expression are unknown, however IL-20 receptor is upregulated in psoriatic epidermis, as well as some leukocytes and endothelial cells.

itself, IL-20 exerts distinctly proinflammatory effects on keratinocytes and may play a central role in the epidermal response to inflammation.

Two functions of the epidermis

When investigating the role of cytokines in the epidermis, we must consider that skin has two fundamental functions: first, it provides a self-repairing physical barrier to keep the rest of the organism in and the outside world out, and second, it acts as a dynamic environment for the front line of the immune system to encounter and respond to pathogens. These two functions act in concert to protect the organism from the environment. Figure 1 illustrates some of the signaling pathways involving the epidermis. Several different circumstances can lead to changes in the homeostasis of the epidermis and structural perturbations. Some phenomena such as physical trauma, ultraviolet radiation or bacterial lipopolysaccharide have direct effects on keratinocytes and underlying fibroblasts, triggering the innate immune system, whereas others such as antigenic challenges are mediated by cells of the acquired immune system.

Differentiation vs proliferation in the epidermis

The outermost layer of skin, the epidermis, consists of layers of keratinocytes stratified in a gradient of differentiation (Figure 1). Keratinocytes progress through a program of differentiation as they move up through the skin to become corneocytes, dead bundles of precipitated keratin proteins wrapped in remnants of plasma membrane. These protein 'bricks' and lipid 'mortar' form the barrier of the skin called the stratum corneum. While cornified cells are continually sloughed off, the less-differentiated keratinocytes of the innermost basal layer of the epidermis proliferate and differentiate as they are forced upwards, continually reforming the barrier. Consequently, the structure of the epidermis is exquisitely dependent upon the homeostatic balance between proliferation and differentiation by the viable keratinocytes in the lower layers of the

epidermis. One of the keys to understanding the biology of the skin is deciphering the molecular events that influence the keratinocytes' decision between proliferation and differentiation. Signals originating in the dermis, including keratinocyte growth factor, are amplified by induced expression of activin, and alter this balance during wound repair and development [8]. IL-20 appears to be a signal originating in the epidermis that directly impacts this decision in the context of inflammation.

Activation of keratinocytes

In addition to altering the balance between proliferation and differentiation, keratinocytes participate in immune responses by releasing signaling molecules. The ability of keratinocytes to release complex arrays of proinflammatory factors when provoked by stimuli such as physical trauma, ultraviolet irradiation, bacterial products or cytokines allows them to recruit inflammatory cells and regulate their behavior. Factors released by keratinocytes in response to various stimuli include TNF-α, IL-1α, IL-3, IL-6, IL-7, IL-8, IL-10, TGF-α, TGF-β, IFN-γ and MCP-1 among others. It is not clear whether these are products of a single type of keratinocyte activation or derive from several qualitatively different responses. In either case, these factors convey signals in a paracrine fashion to other cells including leukocytes, endothelial cells and fibroblasts as well as in an autocrine fashion to the keratinocytes themselves. Two prominent autocrine factors for keratinocytes, TNF-α and IL-1α, are termed primary cytokines because they activate the NFκB pathway, stimulating transcription of a number of proinflammatory cytokines, chemokines, adhesion molecules and other effectors in many cell types.

The biology of IL-20 must be viewed within the context of this already crowded milieu of intercellular signals involved with cutaneous inflammation and homeostasis. Among these signals, however, there is no clear pathway by which activated T cells can trigger the release of primary cytokines by keratinocytes and the ensuing cascade of proinflammatory events in the skin. The prominent expression of the IL-20 receptor in psoriatic skin hints that IL-20 expression may be an important step in this process.

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nonblocking antibody BV12 recognizes both. Thus, dimerization and homophilic binding might contribute to both the junctional localization and adhesive function of JAM-1.

Claudins

Extremely complex TJs are found between cerebral endothelial cells that form the blood–brain barrier (BBB)[8]. Britta Engelhardt (Bad Nauheim, Germany) explained that during inflammation, such as in experimental autoimmune encephalomyelitis (EAE), the specialized properties of the BBB are frequently lost, resulting in brain edema. In EAE, the specific loss of immunostaining for the TJ molecule claudin-1 from inflamed cerebral vessels was observed, whereas the localization of the endothelial-specific claudin-5 and other TJ molecules, such as occludin and ZO-1, was conserved. In an *in vitro* model of the BBB, this selective loss of claudin-1 correlates directly with the opening of endothelial TJs (Fig. 1). These data demonstrate that claudin-1 plays a central role in determining the permeability of TJs of the BBB.

Conclusions

It is an old, but ever more valuable, concept that interfering with the functions of vascular cells and/or leukocytes can stop the progression of chronic inflammatory diseases. Although existing drugs block some aspects of the inflammatory mechanisms, they lack specificity and produce side-effects. Thus, new therapeutic targets in vascular biology are necessary to allow the development of better and more-specific therapies. The above examples suggest several new mechanisms involved in inflammatory diseases. Understanding the molecules and signal-transduction pathways that regulate vascular cell–cell junctions, vascular permeability and the transmigration of leukocytes will point to novel therapeutic targets for the specific inhibition of inflammatory diseases.

Acknowledgements

The cited participants of the meeting contributed actively to this report. We regret that, owing to space constraints, the text represents only a small selection of the presentations.

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IL-10 and its homologs: important immune mediators and emerging immunotherapeutic agents

Hans-Dieter Volk, Khusru Asadullah, Grant Gallagher, Robert Sabat and Gerald Grütz

The 3rd International Workshop on IL-10 and Related Molecules was held at Charité Berlin, Germany from 4–7 April 2001.

In 1989, Mosmann and colleagues first described a cytokine that is produced by T helper 2 (Th2)-cell clones and inhibits the synthesis of interferon γ (IFN-γ) by Th1-cell clones[1]. Today, this 'cytokine synthesis inhibiting factor' (CSIF) is known as interleukin-10 (IL-10), and a capacity to produce IL-10 has been demonstrated for various cell populations, including certain T-cell subsets [e.g. Th2 and T cytotoxic 2 (Tc2) cells], B cells, monocytes, macrophages and keratinocytes. IL-10 was considered to be a purely deactivating, immunosuppressive and anti-inflammatory cytokine initially, but recent investigations have demonstrated more-complex characteristics. In addition, novel homologs of IL-10, derived from mammalian as well as viral genomes, have been described.

The role of IL-10 in disease and its use as a therapeutic agent

Owing to its anti-inflammatory and immunosuppressive properties, IL-10 became a candidate for the therapy of several immunological diseases characterized by a Th1-type cytokine pattern, such as inflammatory bowel disease, rheumatoid arthritis, psoriasis and transplant rejection. Overall, early Phase II clinical trials using recombinant human IL-10 have demonstrated the safety and efficacy of this therapy for all of these indications. However, whereas a positive response to treatment with IL-10 was observed for psoriasis (K. Asadullah, Berlin and K. Reich, Goettingen, Germany), psoriatic arthritis did not improve considerably (I. McInnes, Glasgow, UK). Interestingly, it was demonstrated that long-term low-dose IL-10 therapy after the clearance of active psoriasis by conventional therapy is very effective at preventing a relapse and prolonging the disease-free interval (K. Asadullah). These data suggest that the immunoregulatory activity of IL-10 (e.g. the long-lasting expansion of the

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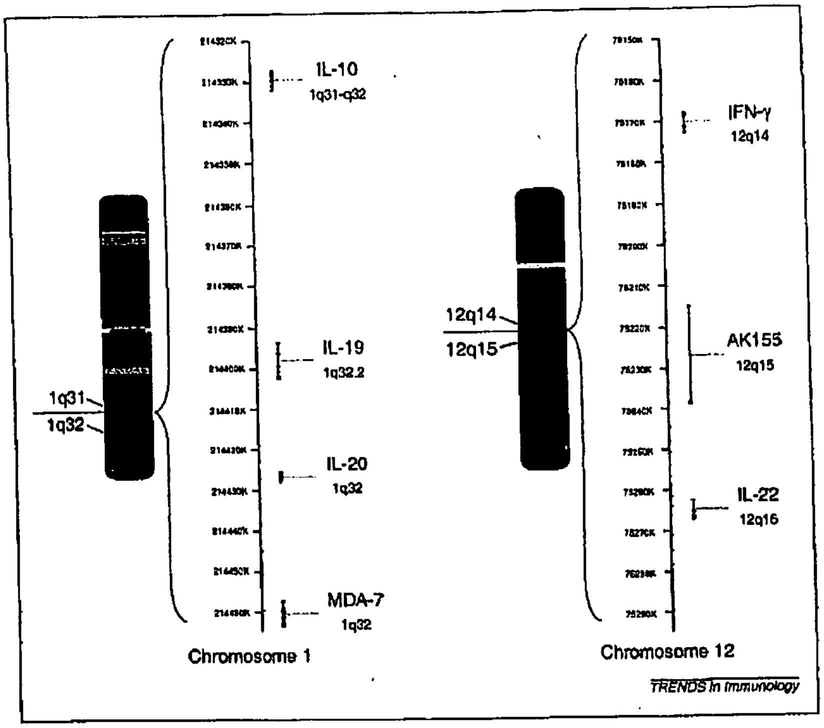

Fig. 1. The genomic localization of the genes encoding interleukin-10 (IL-10), interferon γ (IFN-γ) and the novel IL-10 homologs on human chromosomes 1 and 12. Abbreviations: AK155, Andrea Knappe 155 gene; MDA-7, melanoma differentiation-associated gene 7.

number of IL-4-producing T cells and decreased levels of CD80/CD86) might be more powerful than its anti-inflammatory properties [e.g. decreased synthesis of tumor necrosis factor (TNF) and/or IFN-γ]. It remains to be determined whether similar prophylactic approaches might be as effective and safe in the treatment of other immune diseases.

The delivery of IL-10 might be improved by gene therapy. Intragraft overexpression of IL-10 by adenoviral gene transfer prolongs graft survival in several models (T. Ritter, Berlin, Germany and S. Qian, Pittsburgh, PA, USA). However, this approach is limited by the high inflammatory potency of adenoviral vectors and the difficulties in generating the desired concentration of IL-10. A novel approach to target IL-10 to the site of undesirable inflammation is the use of polyclonally stimulated or antigen (Ag)-specific T-cell lines (C. van Montfrans, Amsterdam, the Netherlands and T. Ritter, respectively) that are retrovirally transduced with IL-10 and express specific tissue-homing receptors.

The role of IL-10 in allergic and atopic disorders seems to be complex. On the one hand, IL-10-mediated peripheral T-cell tolerance induced by allergen-specific immunotherapy, as well as by exposure to natural Ags, is considered to play a key role in the control of specific immune responses to high doses of Ag and/or allergen (C. Akdis, Davos, Switzerland). On the other hand, a lack of effectiveness of systemic IL-10 therapy was clearly demonstrated for atopic dermatitis (K. Reich). This might point to distinct underlying immunological mechanisms in the different types of allergies and atopic disorders.

It has been shown that IL-10 plays an important role in the pathogenesis of post-traumatic immune deficiency. Systemic inflammation is counter-regulated by the release of IL-10 and other immunosuppressive cytokines that are important to prevent septic shock. This counter-regulation is realized at the level of the macrophage but also the neuro–immune pathways, including the sympathetic nervous system, the vagal nerve and the hypothalamic-pituitary–adrenal axis (S. Rupprecht, Berlin, Germany). Excessive counter-regulation can lead to impairment of the immune system ('immunoparalysis') contributing to the high rate of late mortality seen in patients in intensive care.

Genetic polymorphisms strongly influence the capacity for the secretion of IL-10 by immune cells. Conflicting data have been published on 'high'- and 'low'-producer genotypes. This might be explained, at least in part, by the observation that specific polymorphisms in the promoter region of the gene encoding IL-10 have different influences on the expression of IL-10 depending on the particular trigger and type of cell involved (R. Kay, Dundee, UK). Several polymorphisms of the gene encoding IL-10 are associated with the incidence and severity of various immune diseases, including autoimmune diseases, transplant rejection, infections and lymphomas (J. Eskdale, Glasgow, UK; S. d'Alfonso, Milano, Italy; J. Cavot, Newcastle, UK; A. Vergopoulos, Berlin, Germany; and J. May, Tuebingen, Germany). The workshop developed a nomenclature for the described polymorphisms to facilitate the comparison of the data (G. Gallagher, Glasgow, UK).

IL-10 and regulatory T cells

The existence of regulatory T cells has been known for several years. However, the subgroup of T cells that these regulatory cells represent and the mechanisms of their inhibitory function remain to be determined. The production of IL-10 or transforming growth factor β (TGF-β) is thought to be important for the generation and/or action of regulatory T cells. The combination of cytokines expressed by regulatory T cells can depend on the manner by which they were generated and the type of dendritic cell (DC) used (M. Grazia-Roncarolo, Milano, Italy and A. Enk, Mainz, Germany). A. O'Garra (Palo Alto, CA, USA) described a completely different approach to the generation of regulatory T cells by treating Ag-presenting cells (APCs) with vitamin D_3 and dexamethasone. The inhibition of activation of nuclear factor κB (NF-κB) in APCs blocks their expression of pro-inflammatory genes and 'danger' signals, which leads to an increased generation of IL-10-producing

Table 1. The properties of IL-10-related molecules[a]

IL-10 homolog	Cellular source	Biological effects: In vivo	Biological effects: In vitro	Receptors
IL-19	Activated monocytes (LPS- or GM-CSF-stimulated)	ND	No effects on cytokine synthesis by PBMCs	ND
IL-20	LPS-stimulated PBMCs and monocytes (?)	Overexpression in mice causes retardation of growth and development, skin abnormalities and neonatal lethality	Enhancement of IL-1β-induced expression of inflammation-related genes (e.g. HaCaT)	IL-20Rα and IL-20Rβ
IL-22	Activated T cells (Th1 ?), IL-9-stimulated mast cells and mesangium cells	Application in adult mice induces an acute phase response and basophilia in proximal renal tubules; it acts as an autocrine factor for mesangium cells	Induction of an acute phase response (hepatoma cell lines), inhibition of IL-4 production by Th2 cells and activation of STAT3 in hepatocytes	IL-22Rα and IL-10Rβ
AK155	T-cell lines (CD4[+] and CD8[+]), herpes virus saimiri-infected T cells and activated monocytes (?)	ND	ND	ND
MDA-7	Melanoma cells, skin fibroblasts and activated PBMCs (LPS- or PHA-stimulated)	Antitumor effects	Irreversible growth arrest of tumor (induction of apoptosis or differentiation), inhibition of angiogenesis and induction of expression of Th1-type cytokines in PBMCs (?)	ND

[a]Abbreviations: AK, Andrea Knappe; GM-CSF, granulocyte–macrophage colony-stimulating factor; IL, interleukin; LPS, lipopolysaccharide; MDA, melanoma differentiation associated gene; ND, not determined; PBMC, peripheral blood mononuclear cell; PHA, phytohemagglutinin; Th, T helper; STAT3, signal transducer and activator of transcription 3.

regulatory T cells in coculture. So far, it has been difficult to propagate these *in vitro*-generated regulatory T cells efficiently. The addition of IL-15 (instead of IL-2) seems to improve the clonal expansion of regulatory T cells (M. Grazia-Roncarolo).

IL-10 signaling and molecular mechanisms of inhibitory function

Although the immunosuppressive effects of IL-10 have been known for some time, there have been conflicting reports about the molecular basis of this inhibition. The nuclear translocation of the transcription factor NF-κB heterodimer is considered to be responsible for most of the transcriptional activity resulting from stimulation with lipopolysaccharide (LPS). The induction of the activity of mitogen-activated protein (MAP) kinases (e.g. p38) might play a role in the stabilization of mRNA or the ease of translation of cytokine mRNAs. Therefore, both NF-κB and MAP kinases are putative targets for the inhibitory action of IL-10; their possible inhibition has been studied by several groups with very different results.

These differences were reflected in talks given at the IL-10 workshop. IL-10 blocks the nuclear translocation of the p65 subunit of NF-κB, whereas the nuclear translocation of the p50 subunit remains intact, leading to the accumulation of inhibitory p50 homodimers in the nucleus (F. Driessler, Berlin, Germany). T. Mijatovic (Brussels, Belgium) demonstrated that in mouse macrophages, IL-10 mainly inhibits the expression of TNF at the post-transcriptional level. The 3′ untranslated region (UTR) of the TNF mRNA (containing AU-rich regions), as well as the 5′UTR, seem to be necessary for the complete inhibitory function of IL-10. That these different observations depend very much on the model used was discussed by B. Foxwell (London, UK). There are obvious differences between cell lines and primary cells. Both transcriptional and post-transcriptional inhibitory mechanisms are employed by IL-10 in human primary monocytes using adenoviral-delivered reporter constructs. The dominant mechanism depends on the time point at which IL-10 is added. If the cells are preincubated with IL-10, transcriptional inhibition dominates, but when IL-10 is added simultaneously with LPS, the 3′UTR is required for inhibition by IL-10, suggesting a post-transcriptional mechanism. Murine macrophages showed only the 3′UTR-mediated inhibition, regardless of the timing of

Key outcomes of the meeting

- IL-10 has been shown to be effective and safe in several clinical trials (e.g. for the treatment of rheumatoid arthritis) although it was less effective than therapy with anti-TNF mAb.
- In the treatment of psoriasis, IL-10 reversed acute disease effectively and prevented the relapse of disease activity. This might represent a novel approach for therapy with IL-10.
- It is possible to expand IL-10-driven regulatory T-cell populations *in vitro* using IL-15, without the loss of their suppressor function.
- IL-10 upregulates the expression of >300 genes, suggesting the complexity of its signaling.
- Several IL-10 homologs have been described. None of these appear to share the immunoregulatory and anti-inflammatory properties of IL-10. However, they have interesting biological activities including: antitumor effects, the induction of expression of acute-phase proteins and the stimulation of keratinocytes to proliferate.

exposure to IL-10, suggesting underlying species-specific differences in the mechanism of inhibition by IL-10.

The activation of signal transducer and activator of transcription 3 (STAT3), which is strongly influenced by signaling through the IL-10 type I receptor (IL-10RI), plays a key role in mediating the effects of IL-10 (M. Cassatella, Verona, Italy and R. de Waal Malevyt, Palo Alto, CA, USA). There is a region in the C-terminus of the IL-10R1 that (in addition to the activation of STAT3) is necessary for the immunosuppressive function of IL-10 in macrophages but dispensable for the activating function of IL-10 in B cells (B. Weaver, St Louis, MO, USA). This region is not involved in activating the Jak–STAT pathway. The Weaver group has identified an IL-10-responsive gene (TIGER), expression of which is dependent on the presence of this C-terminal region. This should help to identify the downstream signaling events.

The hunt is now on to find those genes that are mediators of the immunosuppressive function of IL-10. Some groups have used differential mRNA analysis to address this question. Preliminary results of some candidate genes were presented (L. Williams, London, UK), but there is a long way to go to prove the function of those genes whose expression is regulated by IL-10 and to understand the way in which they act at the molecular level.

IL-10-related molecules

During recent years, molecules with homology to IL-10 have been described, mostly as a result of *in silico* work. At the workshop, an update of this interesting group of molecules encoded by mammalian or viral genomes was given by several groups (R. de Waal Malevyt; G. Gallagher; S. Kotenko, New Jersey, NJ, USA; S. Chada, Houston, TX, USA; Y. Chandrasekher, Seattle, WA, USA; E. Caudell, Houston, TX, USA; J. Peat, Glasgow, UK; J-C. Renauld, Brussels, Belgium; L. Fouser, Cambridge, MA, USA; and H. Fickenscher, Erlangen, Germany). The human homologs are located on chromosomes one and 12, close to the gene loci encoding IL-10 and IFN-γ, respectively (Fig. 1). Three of these homologs [IL-19, IL-20 and melanoma differentiation-associated antigen 7 (MDA-7)] are produced by stimulated peripheral blood mononuclear cells[2]. None of the related molecules seem to possess the inhibitory activities of IL-10, but their strong antitumor and selective pro-inflammatory activities make them interesting new candidates for research and drug development. Table 1 summarizes the most important properties of these homologs.

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Keeping up with the *Trends*

Articles of interest to immunologists in other *Trends* journals:

Arroyo, J. *et al.* (2001) Yellow fever vector live-virus vaccines: West Nile virus vaccine development. *Trends Mol. Med.* 7, 350–354

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Anti-inflammatory

IL-20: a new target for the treatment of inflammatory skin disease

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The discovery of dramatic pro-inflammatory effects of IL-20 on skin highlighted a novel regulatory pathway in cutaneous inflammation. Specific receptor complexes for IL-20 are induced on keratinocytes and transmit potent signals via the signal transducer and activator of transcription-3. In response to IL-20, keratinocytes proliferate and express pro-inflammatory genes including TNF-α, which leads to activation of NF-κB. Recently, two related cytokines, IL-19 and IL-24, have been shown to trigger the IL-20 receptor, and a second receptor complex has also been found to respond to IL-20 and IL-24. IL-20 signalling appears to be a prominent component of cutaneous inflammation, but the extent to which inflammatory processes rely upon it is unknown. Nonetheless, the prevalence of diseases involving pathological cutaneous inflammation makes the identification of safe and effective anti-inflammatory therapies for the skin a priority. Detailed understanding of the signal transduction pathways by which the skin responds to IL-20 and related factors may make it possible to develop new pharmaceutical agents to selectively suppress cutaneous inflammation.

Keywords: cytokine, epidermis, IL-19, IL-20, IL-22, IL-24, IL-26, inflammation, keratinocyte, receptor, skin, signal transducer and activator of transcription-3 (STAT-3)

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1. Introduction

Coordination of the action of cells and tissues of multicellular organisms requires cell–cell communication. One of the prominent mechanisms by which cells transmit signals to other cells is by the release of molecules, including soluble proteins known as cytokines, which bind to specific receptors and provoke changes in target cells. Some of these signalling events are associated with developmental or homeostatic processes while others are part of reactive immune responses. A number of disease conditions involve inappropriate or excessive inflammatory reactions. An understanding of the molecular mechanisms of these reactions can lead to the development of hypothesis-based interventions. The characterisation of IL-20 and the discovery of its potent effects on keratinocytes has revealed a new regulatory circuit contributing to cutaneous inflammation. More recently, IL-19 and IL-24 have been shown to engage and activate the IL-20 receptor, and a second receptor complex that responds to IL-20 and IL-24 has been identified. In this review, the signalling pathways of IL-20 and related cytokines will be examined in the context of the normal biology and pathological reactions of the skin.

2. Skin inflammation in disease

The skin is the largest and most visible organ of the body, and its health is both a critical component of quality of life and an important gauge of overall health. In addition

[illegible] effects, the emotional toll of chronic dis- [illegible] changes in appearance and disfigurement caused [illegible] skin disorders, can be very heavy. The prevalence of [illegible] disorders and the imperative to control them combine to create a significant demand for effective interventions.

Many of the pathological conditions of the skin are caused by inflammatory reactions of the immune system. The most frequent skin disorders, dermatophytoses and acne, involve inflammatory responses provoked by pathogens (fungi and bacteria, respectively). Inflammation associated with these conditions subsides when the infection is controlled by antibiotics or prevented by altering the behaviour of sebaceous glands. Several other skin disorders involving inflammation of unknown aetiology affect as many as 5% of the US population. These include seborrheic dermatitis, atopic dermatitis and psoriasis. Of these, expenditures on medications to treat psoriasis are disproportionately high, largely because of its persistence and resistance to treatment [1].

Psoriasis is a disorder that presents as patches of crusty scales over glossy reddened skin. The most prominent feature of psoriasis is the hyperproliferation and perturbed differentiation of epidermal keratinocytes leading to accumulation of scales. Abnormal angiogenesis and infiltration of inflammatory T_{H1} cells expressing IFN-γ are also evident. In the past, the epidermal hyperproliferation has been ascribed to cell-autonomous defects in the keratinocytes. More recently, it has become clear that activation of T cells is a critical component of the pathogenic process. Several biological agents that target pathogenic T cells have been found to be effective in controlling psoriasis. These include an anti-CD4 monoclonal antibody [2,3], an IL-2 diphtheria toxin fusion protein [4], an anti-CD11a monoclonal antibody [5], and a fusion protein consisting of lymphocyte function-associated antigen-3 (LFA-3) and the Fc domain of immunoglobulin [6]. The therapeutic effects of the potent immunosuppressive drugs cyclosporin A [7] and FK506 (tacrolimus) [8] in the treatment of psoriasis have been known for some time. While these drugs are best known for their effects on T cells, they have also been demonstrated to act directly on keratinocytes [9-11].

In contrast to psoriasis, atopic dermatitis (also known as atopic eczema) is an allergic disorder that frequently arises in individuals who have, or will develop allergic rhinitis or constrictive airway disorders (asthma). Atopic dermatitis is associated with elevated levels of IgE and enhanced expression of T_{H2} cytokines IL-4, IL-5 and IL-13. It involves chronic activation and degranulation of mast cells and vascular permeability leading to oedema.

Thus, psoriasis exhibits features of a cell-mediated Type 1 immune reaction, while atopic dermatitis more closely resembles a humoral or Type 2 immune reaction.

3. The active role of skin

3.1 Differentiation versus proliferation in the epidermis

As the interface with the outside world, the primary function of the skin is to act as a protective barrier. It keeps foreign material out of the body and keeps moisture in. The epidermis maintains the barrier by undergoing a continuous process of regeneration in which proliferation of the basal layer of keratinocytes adjacent to the dermis pushes cells out to the surface as they differentiate and die. The precipitated structural proteins of the cells (keratins), interspersed with the hydrophobic components of the membranes, adhere together to form the impermeable barrier known as the stratum corneum. As these cornified cells are broken loose by abrasion, they are replaced by new cells from below.

The structure of the epidermis is determined by this homeostatic balance of proliferation, differentiation and sloughing. Perturbations in the behaviour of keratinocytes can have a dramatic impact on the health of skin. Enhanced keratinocyte proliferation can result in a significant thickening of the epidermis termed acanthosis. Excessive proliferation also pushes some keratinocytes up into the stratum corneum before they fully differentiate. This phenomenon, called parakeratosis, is characterised by defects in the barrier function and retention of nuclear remnants in the stratum corneum.

Understanding the mechanism by which a keratinocyte decides between differentiation and further proliferation is an important goal of cutaneous research. During development and wound repair, this balance is affected by signals transmitted from the dermis [12-14]. This balance is also affected by inflammation, and IL-20 may be a signal originating from within the epidermis that contributes to this decision.

3.2 Keratinocyte activation

The secondary function of the skin is to interact with the immune system. The epidermis acts as a sensor for pathogenic and traumatic challenges. In response to those challenges, it transmits pro-inflammatory signals to other resident cutaneous cells as well as more mobile cells of the haematopoietic immune system. Certain stimuli have direct effects on keratinocytes and underlying fibroblasts causing them to release cytokines that trigger the innate immune system. The ability of keratinocytes to release complex arrays of pro-inflammatory factors when provoked by stimuli such as physical trauma, ultraviolet irradiation, bacterial products or cytokines, allows them to recruit inflammatory cells and regulate their behaviour.

Factors released by keratinocytes in response to various stimuli include: TNF-α, IL-1α, IL-3, IL-6, IL-7, IL-8, IL-10, IL-12, IL-15, IL-18, TGF-α, TGF-β, IFN-γ and monocyte chemotactic protein-1 (MCP-1), among others [15]. Although it is not yet clear under which circumstances keratinocytes express IL-20, it appears that it should now also be included in this list. These factors convey signals in a paracrine fashion to other cells including leukocytes, endothelial cells, fibroblasts and adjacent keratinocytes as well as in an autocrine fashion to the activated cells themselves. As discussed below, TNF-α, IL-1α, and IL-18 are three prominent autocrine factors that are important for amplifying and sustaining the activated state of the keratinocytes; they also

transmit signals to many other types of cells. IL-20 appears to be both an autocrine and paracrine factor that amplifies and sustains keratinocyte activation.

Other stimuli, such as antigenic challenges, are mediated by cells of the acquired immune system. Foreign antigens are captured and processed by antigen-presenting cells (APCs) and carried to lymphoid tissue where they activate antigen-specific T cells. Antigen-specific T cells migrate into the tissue where they encounter more antigen and become further activated. Products released by activated T cells convey signals to keratinocytes. IFN-γ, produced by T_{H1} cells, stimulates keratinocytes to express intercellular adhesion molecule-1 (ICAM-1), which facilitates adhesion to lymphocytes, and IL-7, which supports viability of T cells. Therefore, in addition to transmitting signals to recruit and activate effector cells, the epidermis undergoes changes to provide a supportive environment for the immune system as it repels pathogens.

4. Primary cytokines and the NF-κB activation cycle

TNF-α, IL-1 and IL-18 have been called primary cytokines because binding to their cognate receptors results in the activation of the transcription factor NF-κB, which in turn activates genes encoding a broad array of pro-inflammatory products [16]. In the absence of activating signals, NF-κB resides sequestered as an inactive form in the cytoplasm bound tightly to a specific inhibitory molecule, Iκβ. Signals transmitted by the primary cytokine receptors lead to degradation of Iκβ and release of the active form of NF-κB. Activated NF-κB translocates to the nucleus where it promotes transcription of IL-1, TNF-α and other pro-inflammatory genes including cellular enzymes, adhesion molecules, chemokines and cytokines. This self-enhancing circuit is a central mechanism in the cellular inflammatory response that provides for prolonged and enhanced activation of the array of genes associated with inflammation (Figure 1).

The NF-κB activation circuit can be triggered by exposure to bacterial products [17], physical stresses such as osmotic shock or ultraviolet irradiation, which directly activate the epidermal growth factor (EGF), TNF and IL-1 receptors [18], or trauma, which releases intracellular stores of preformed IL-1α [19]. The ability of glucocorticoids to inhibit the activation and action of NF-κB contributes to their broad anti-inflammatory properties [16,20]. Recently developed antagonists of the TNF-α [21] and IL-1 [22] receptors also exert potent anti-inflammatory effects by disrupting the NF-κB activation cycle.

The discovery that signalling by the IL-20 receptor activates the TNF-α gene in keratinocytes identifies a previously unknown mechanism of initiating the NF-κB activation circuit. While the activation of NF-κB by increased expression of TNF-α is likely to contribute to the dramatic phenotype of IL-20 transgenic mice, the normal biological circumstances in which this is relevant are unclear [23].

5. IL-20 as an autocrine factor for keratinocytes

IL-20 was initially identified as a mRNA expressed in keratinocytes that is related to IL-10 and contains instability sequences in it's 3′ untranslated region [23]. The relevance of IL-20 to the skin was first appreciated when several transgenic mice were generated with a cDNA encoding IL-20 driven by various promoters. Each of these mice were runted, had abnormally tight and shiny skin and died within days of birth. The epidermis of the transgenic mice was found to be profoundly thickened and had increased numbers of keratinocytes. Moreover, expression of certain markers of differentiation and proliferation, which are normally confined to the basal layer, was detected in the suprabasal layers of the epidermis. These include proliferating cell nuclear antigen (PCNA) and keratins-5, -6 and -14 [23]. These perturbations appeared to have been caused by circulating IL-20, since the phenotype was observed in mice that expressed the transgene in tissues other than skin, such as in the liver from the albumin promoter, as well as in mice with a transgene directed to skin.

The profound effects of IL-20 on the epidermis of the transgenic mice, and the fact that IL-20 was found to be expressed by keratinocytes, focused attention on IL-20 as an autocrine factor for keratinocytes. Exposure of an immortalised keratinocyte cell line (HaCaT) to IL-20 was found to activate signal transducer and activator of transcription-3 (STAT-3), one of the transcriptional activators involved in IL-10 signalling [23]. Numerous different signalling molecules can activate STAT-3 in several different cell types [24]. Activated STAT-3 translocates to the nucleus and stimulates transcription of several STAT-responsive genes. The central importance of STAT-3 is demonstrated by the observation that STAT-3-deficient mice die as early embryos. Specific deletion of the STAT-3 gene in keratinocytes blocks their responses to EGF, hepatocyte growth factor (HGF) and IL-6 [25]. Although STAT-3-deficient keratinocytes form relatively normal skin, they are defective in wound healing and have altered secondary hair cycles. While EGF is clearly an important cytokine for keratinocytes, it is possible that some of the deficits in STAT-3-deficient keratinocytes are also due to their inability to respond to IL-20. IL-10 signal transduction includes both STAT-3-dependent and -independent pathways [26]. Therefore, it is possible that IL-20 signal transduction may also utilise multiple pathways. As discussed below, two different receptor complexes are triggered by engagement of IL-20, but each of these activates STAT-3 [27].

6. Cytokines related to IL-20

6.1 Genetics

IL-20 is one of at least a dozen cytokines related to IL-10 [28]. Six members of the IL-10 family are encoded by genes at two loci in the human genome. Genes coding for IL-10, IL-19, IL-20 and IL-24 (formerly melanoma differentiation-associated

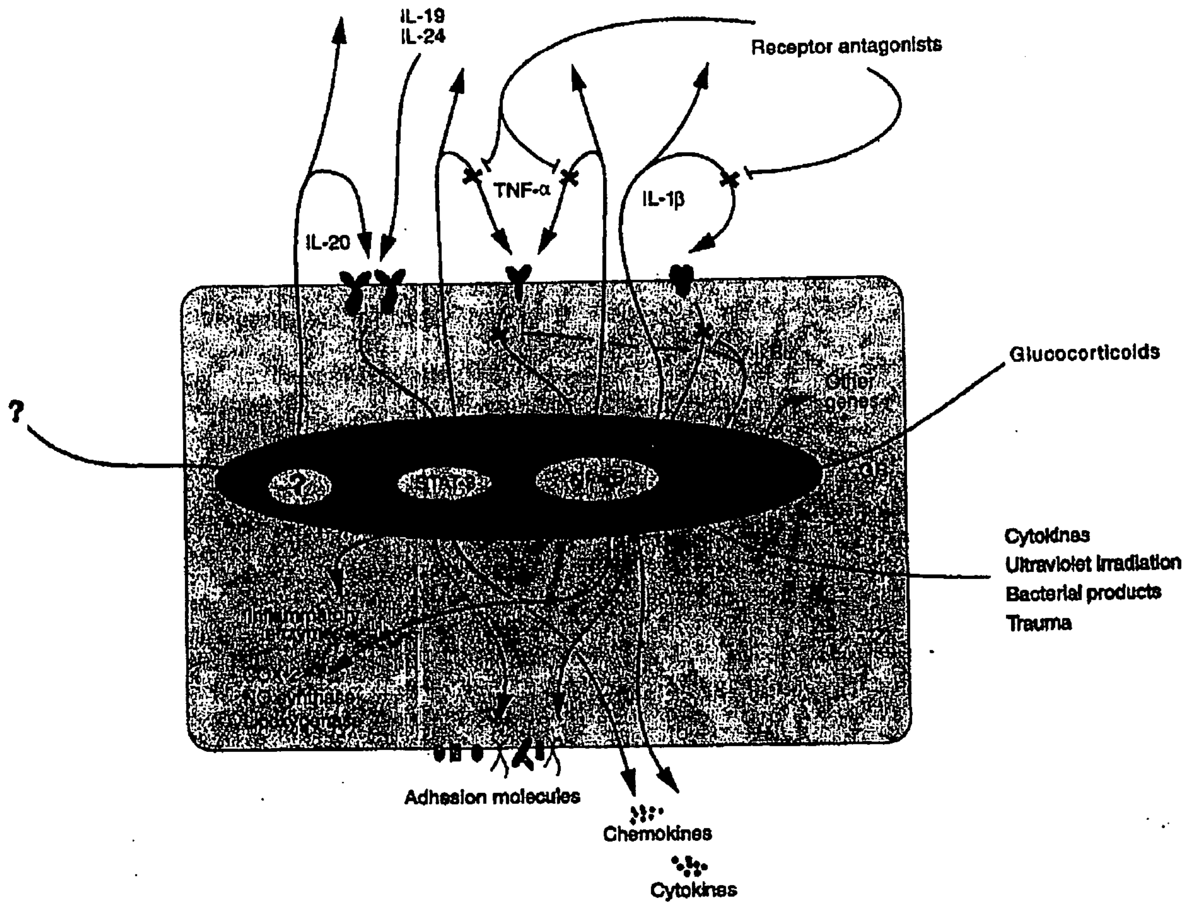

Figure 1. IL-20 and NF-κB activation in keratinocytes. Engagement of the TNF-α and IL-1 receptors activates NF-κB, which in turn stimulates further expression of IL-1 and TNF-α and an array of other pro-inflammatory genes. This amplification cycle can be triggered by direct stimuli, such as bacterial products, ultraviolet irradiation or trauma, which releases intracellular stores of preformed IL-1. IL-20 also contributes to the activation of NF-κB by turning on TNF-α and other pro-inflammatory genes. IL-1 further enhances the sensitivity of keratinocytes to IL-20 and amplifies their response to it. Glucocorticoids exert their broad immunosuppressive effects by altering the expression of numerous genes including upregulation of IκBα, which blocks the activation and action of NF-κB, and downregulation of NF-κB itself. IL-1 and TNF-α receptor antagonists diminish NF-κB activation by competing for extracellular ligands.
COX: Cyclooxygenase; GR: Glucocorticoid receptor; NO: Nitrogen oxide; STAT: Signal transducer and activator of transcription.

gene-7 [MDA-7], mob-5, IL-4-induced secreted protein [FISP], C49a, respectively) are clustered on chromosome 1 near position 1q32 [23,29,30], while the genes coding for IL-22 and IL-26 (formerly IL-10-related T cell-derived inducible factor [IL-TIF] and AK-155, respectively) are near one another in the vicinity of the gene for IFN-γ on chromosome 12 at position 12q15 [31,32]. It is clear that these genes evolved from a common ancestor, and the fact that they are found clustered together may indicate that they have evolved relatively recently by duplication events. Interestingly, the murine IL-22 gene, which is also close to the IFN-γ gene, is duplicated in some strains of mice [32].

At least seven other IL-10-related cytokines are encoded by various viral genomes [28]. The presence of these IL-10-related genes in viral genomes is a clear indication that their bioactivities as agonists (engaging and triggering cognate receptors) or antagonists (non-signalling competitive inhibitors), confer competitive advantages to the viruses, most likely by interfering with host immunity. The ability of IL-10 to reign in inflammatory immune responses is also exploited by certain intracellular bacteria. While they do not produce their own versions of IL-10, they provoke expression of high levels of endogenous IL-10 by host cells [33].

6.2 Biological activities

While some constitutive expression of IL-10, IL-19 and IL-24 has been detected, the IL-10 family of cytokines are principally

expressed in response to activating stimuli. The six IL-10-related cytokines encoded by the human genome are produced by different cells in response to various stimuli, and appear to have diverse biological roles.

IL-10 is a product of certain activated T cells, B cells, monocytes and keratinocytes, and diminishes activation and effector functions of T cells, monocytes and macrophages while promoting the production and function of T_{H2} and regulatory T cells. The biology of IL-10 has been studied extensively and is reviewed elsewhere [34].

IL-19 is a product of B cells and monocytes stimulated with lipopolysaccharide (LPS) or granulocyte macrophage colony-stimulating factor (GM-CSF) [30,35]. Exposure of monocytes to IL-19 induces them to release IL-6 and TNF-α, produce reactive oxygen compounds, and undergo apoptosis [36]. In contrast to IL-20, transgenic mice expressing IL-19 are reported to lack any obvious cutaneous pathology [37].

IL-22 is produced by T cells and natural killer (NK) cells in response to IL-9, and elicits an acute phase response from the liver [38-40] and inflammatory changes in the pancreas [41]. Structural analysis of IL-22 reveals that it is distinct from IL-10, and suggests that it may be active as a monomer [42].

IL-24 was originally identified as an autocrine differentiation factor regulated by ras and mitogen-activated protein kinase (MAPK) in melanoma cells [43,44]. It is also produced in healing wounds [50], activated T_{H2} cells [51], peripheral blood mononuclear cells activated by concanavalin A [52], LPS or phytohaemaglutination antigen [53], and macrophages in response to LPS and IL-4, or infection by influenza virus [54]. The ability of IL-24 to induce apoptosis in malignant cells [45,46] has prompted efforts to develop it as a therapeutic anticancer agent [47-49].

IL-26 was identified as a secreted product of T cells, transformed by *Herpesvirus saimiri*, which has structural homology with IL-10, and is encoded by a gene in close proximity to the IL-10, IL-19 and IL-20 genes [31,32,55]. No bioactivities or cognate receptors for IL-26 have been described.

6.3 Receptors and signal transduction

To date, five of the six human IL-10-related cytokines have been shown to engage and trigger one or more of four different dimeric receptor complexes with shared chains. Figure 2 depicts cellular sources and models of the known receptor complexes and signalling pathways for the IL-10 family of cytokines. Like their cognate ligands, expression of the IL-20Rα and IL-20Rβ chains are strongly induced by cellular activation, and they are only detected on keratinocytes, endothelial cells and certain monocytes in association with inflammatory conditions such as psoriasis [23]. As shown in Figure 2, IL-20 and IL-24 can each bind and trigger both the IL-20Rα/IL-20Rβ and the IL-22Rα/IL-20Rβ complexes, while IL-19 only activates the IL-20Rα/IL-20Rβ complex [23,27,37,52]. Engagement of each of these receptor complexes results in the activation of STAT-3 by phosphorylation [23,27,37].

IL-22 engages a receptor consisting of IL-22Rα and IL-10Rβ, which activates Janus kinase-1 (JAK-1) and tyrosine kinase-2 (TYK-2) leading to STAT-1, STAT-3 and STAT-5 phosphorylation and translocation [38-40]. IL-22 signal transduction is similar to IL-10 in that it involves activation of JAK-1 and TYK-2, but it is distinct in that it also activates components of the MAPK pathways that are not activated in response to IL-10. IL-22 signalling also results in phosphorylation of Ser 727 of STAT-3, a feature of IL-6 signal transduction that is not found in IL-10 signalling [56].

7. Pharmaceutical intervention in the IL-20 pathway

7.1 The utility of blocking IL-20 signalling

The response of the epidermis to inflammatory processes contributes to the underlying pathology of many skin diseases. A number of potent drugs with broad immunosuppressive effects, such as corticosteroids, fusion toxins or receptor antagonists, are currently available, and are effective in controlling the symptoms of these disorders, although the adverse consequences of systemic immunosuppression often limit or preclude their clinical use.

Three observations point towards a central role for IL-20 and related cytokines in the regulation of cutaneous inflammation and the ensuing pathological epidermal changes:

- The profound effects of IL-20 expression in transgenic mice.
- The expression of the IL-20R chains in psoriasis.
- The pro-inflammatory effects of IL-20 on keratinocyte gene expression.

The apparently specialised role of IL-20 signalling in cutaneous tissue may present an opportunity to create pharmaceutical interventions that selectively mitigate inflammatory processes in the skin while sparing inflammation in other tissues.

To evaluate prospects for inhibiting biological responses to IL-20, it is helpful to consider our current understanding of the stepwise events involved. IL-20 expression is regulated by unknown mechanisms: released IL-20 diffuses to engage receptors on recipient cells; bound receptors undergo changes that promote activity of cytoplasmic JAK kinases; and cytoplasmic kinases phosphorylate STAT molecules, which then translocate to the nucleus and initiate transcription of certain responsive genes. Biochemical intervention may be possible at one or more components in each of these stages, but only a few approaches have been successful to date.

7.2 Endogenous regulators of signal transduction

Several extracellular and intracellular mechanisms of negative regulation of signalling of various cytokines have been identified. Certain soluble isoforms or homologues of receptors act as binding proteins, scavenging certain cytokines and inhibiting them from binding cell-bound signalling receptors. Consistent with this paradigm, a soluble protein related to the

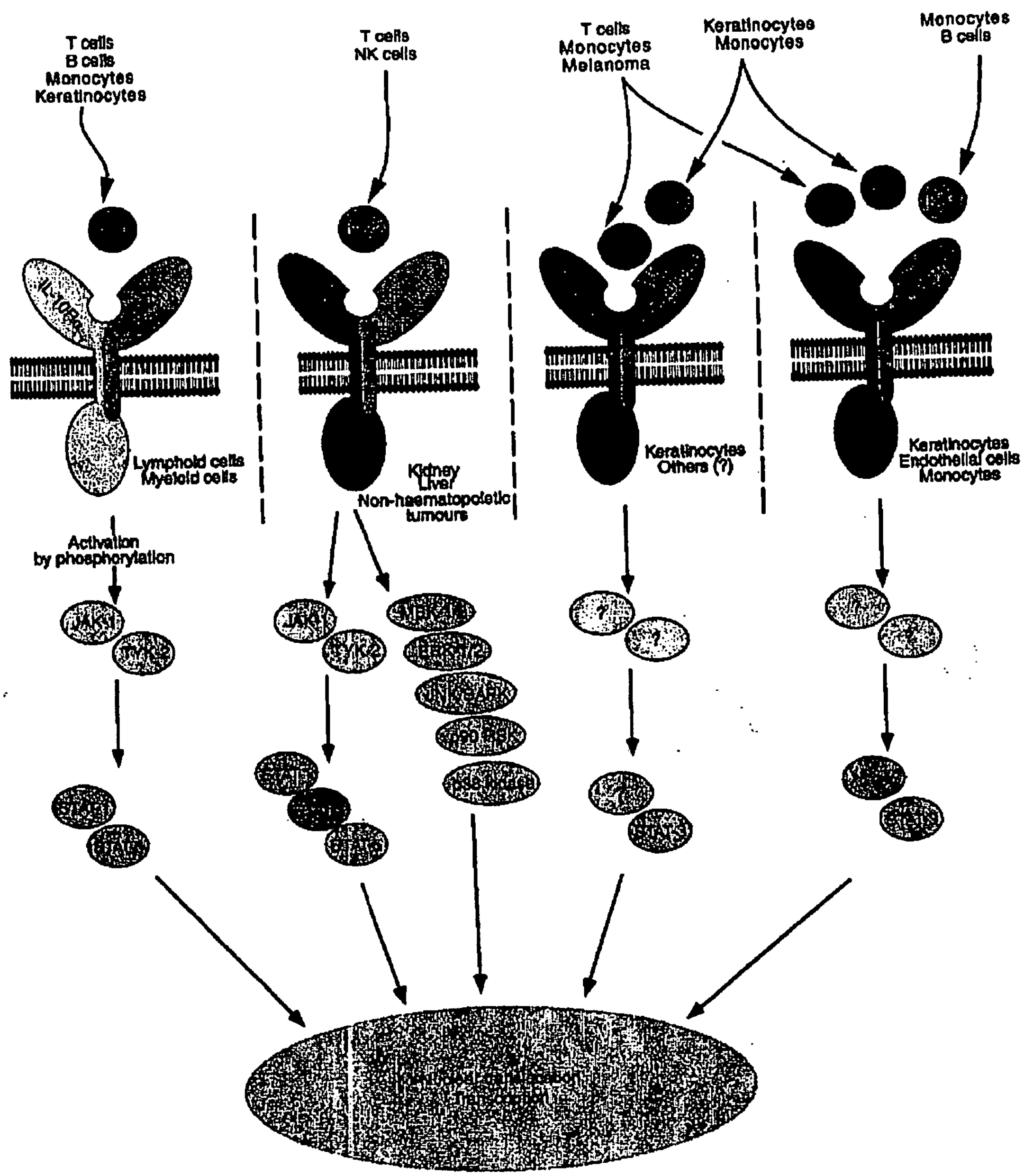

Figure 2. IL-10 family of cytokines and their cognate receptors. Cytokines of the IL-10 family are produced by several different cell types in response to stimuli. Four different receptor complexes, expressed by various cell types, transmit signals in response to cytokines of the IL-10 family. Each complex consists of two transmembrane polypeptides, a larger α-chain with a prominent cytoplasmic domain and a smaller β-chain with a minimal cytoplasmic domain. Upon engagement, the receptor complexes activate JAK proteins, which in turn activate STAT proteins. Activated STATs translocate to the nucleus and regulate transcription of genes. IL-22 has also been shown to activate components of the MAPK signalling pathway and stimulate a serine phosphorylation of STAT-3.

ERK: Extracellular signal-regulated kinase; JAK: Janus kinase; JNK: c-Jun N-terminal kinase; MAPK: Mitogen-activated protein kinase; MEK: Mitogen-induced extracellular kinase; NK: Natural killer; RSK: Ribosomal S6 kinase; SAPK: Stress-activated protein kinase; STAT: Signal transducer and activator of transcription; TYK: Tyrosine kinase.

IL-22 receptor has been described and demonstrated to inhibit the action of IL-22 *in vitro* [57,58].

Eight suppressors of cytokine signalling (SOCS) proteins have been identified that are expressed in response to various cytokines. SOCS proteins are negative regulators of JAK kinases that inhibit their kinase activities and accelerate their ubiquitination and degradation [59]. Protein inhibitors of activated STATs (PIASs) provide another level of regulation of cytokine signalling [60].

It is likely that IL-20 signalling is regulated by some or all of these mechanisms, which may present opportunities for engineering therapeutic inhibitors.

7.3 Modified receptor proteins and antibodies

Of the endogenous mechanisms of controlling cytokine signalling, only extracellular competitive inhibitors constructed from soluble versions of cognate receptors or monoclonal antibodies have been used successfully. The most notable of these are soluble versions of the TNF and IL-1 receptors, as discussed above. Therefore, soluble forms of the IL-20 and IL-24 receptors and monoclonal antibodies are probably the most specific and readily available prospects for blocking the effects of IL-20.

7.4 Small molecule kinase inhibitors

While the specific roles of the various JAK and STAT molecules in IL-20 signal transduction remain to be fully understood, advances in the biochemistry and structure of protein kinases and methods of drug discovery continue to lead to highly selective and effective inhibitors with favourable pharmacokinetics [61-65]. The recent success of the ABL (cellular homologue of Abelson virus kinase) kinase inhibitor STI-571 (imatinib mesylate, Gleevec™, Novartis) in controlling certain myeloid and lymphoid leukaemias demonstrates the promise of this approach [66]. The IL-20 signalling apparatus utilises JAK and STAT molecules that are also necessary for other essential signalling pathways [67]. STAT-3 and JAK-1 are required for signalling in response to IL-10 and IFNs [24,68] and it is likely that they are also required for IL-20 signalling. They are also critical for other important processes, as demonstrated by the fact that STAT-3-deficient mice die as early embryos [69] and JAK-1-deficient mice die soon after birth, probably as a result of aberrant neurological development caused by deficient LIF (leukaemia inhibitory factor) and CNTF (ciliary neurotrophic factor) signalling [70]. TYK-2 is activated in response to IL-10 and IL-22 and perhaps also by IL-20. TYK-2 does not appear to be essential for IL-10 signal transduction, since cells from TYK-2-deficient mice are able to respond normally to IL-10 [71]. Thus, JAK-1 is the kinase most likely to be an effective target for blocking IL-20 signalling, although it may lead to undesirable side effects.

7.5 Ligand–toxin fusion proteins

The highly specific high-affinity interaction between cytokines and their cognate receptors has been exploited to create selective cytotoxic fusion proteins that kill cells bearing the receptors. The most established of these is the IL-2 diphtheria toxin, denileukin diftitox (Ontak®, Ligand Pharmaceuticals, Inc.), for the treatment of psoriasis [4] and cutaneous T cell lymphoma [72]. This approach is best suited to circumstances in which the targeted cognate receptor is only expressed by the pathological cells, as appears to be the case for the IL-20 receptor in psoriasis. On the other hand, killing large numbers of the activated keratinocytes might significantly compromise the skin and impair wound healing.

7.6 Gene expression modifiers

Several schemes have been devised for diminishing the expression of specific genes, including the use of antisense molecules [73] and RNA interference [74]. Agents such as these that target expression of ligands (IL-19, IL-20 and IL-24) or receptors (IL-20α, IL-20β and IL-22α) might be effective in controlling cutaneous inflammation. Conversely, selectively increasing expression of SOCS or PIAS genes in basal keratinocytes or other responsive cells might also diminish IL-20 signalling.

8. Conclusion and expert opinion

The engagement of the IL-20 receptor on keratinocytes has been demonstrated to provoke skin pathology. Although the specific role of IL-20 in inflammation remains uncertain, it appears likely that its expression in keratinocytes participates in the initiation and perpetuation of the NF-κB activation circuit. Furthermore, the expression of ligands for the IL-20 and IL-24 receptors by activated monocytes and some lymphocytes may constitute a previously unknown connection between the haematopoietic immune system and the epidermis. Therefore, the relatively specialised role of IL-20 signalling in cutaneous inflammation may present opportunities for selective therapeutic intervention in cutaneous inflammatory disorders.

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